



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number: PCT/US00/13975</b> <b>(22) International Filing Date: 19 May 2000 (19.05.00)</b>  <b>(30) Priority Data:</b> <table border="0"> <tr> <td>60/134,949</td> <td>19 May 1999 (19.05.99)</td> <td>US</td> </tr> <tr> <td>60/144,270</td> <td>15 July 1999 (15.07.99)</td> <td>US</td> </tr> <tr> <td>60/146,700</td> <td>30 July 1999 (30.07.99)</td> <td>US</td> </tr> <tr> <td>60/157,508</td> <td>4 October 1999 (04.10.99)</td> <td>US</td> </tr> </table> <b>(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications</b> <table border="0"> <tr> <td>US</td> <td>60/144,270 (CIP)</td> </tr> <tr> <td>Filed on</td> <td>15 July 1999 (15.07.99)</td> </tr> <tr> <td>US</td> <td>60/146,700 (CIP)</td> </tr> <tr> <td>Filed on</td> <td>30 July 1999 (30.07.99)</td> </tr> <tr> <td>US</td> <td>60/157,508 (CIP)</td> </tr> <tr> <td>Filed on</td> <td>4 October 1999 (04.10.99)</td> </tr> <tr> <td>US</td> <td>60/134,949 (CIP)</td> </tr> <tr> <td>Filed on</td> <td>19 May 1999 (19.05.99)</td> </tr> </table> <b>(71) Applicant (for all designated States except US):</b> INCYTE GENOMICS, INC. [US/US]; 3160 Porter Drive, Palo Alto, CA 94034 (US).		60/134,949	19 May 1999 (19.05.99)	US	60/144,270	15 July 1999 (15.07.99)	US	60/146,700	30 July 1999 (30.07.99)	US	60/157,508	4 October 1999 (04.10.99)	US	US	60/144,270 (CIP)	Filed on	15 July 1999 (15.07.99)	US	60/146,700 (CIP)	Filed on	30 July 1999 (30.07.99)	US	60/157,508 (CIP)	Filed on	4 October 1999 (04.10.99)	US	60/134,949 (CIP)	Filed on	19 May 1999 (19.05.99)	<b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> TANG, Y., Tom [CN/US]; 4230 Ranwick Court, San Jose, CA 95118 (US). YUE, Henry [US/US]; 826 Lois Avenue, Sunnyvale, CA 94087 (US). LAL, Preeti [IN/US]; 2382 Lass Drive, Santa Clara, CA 95054 (US). BURFORD, Neil [GB/US]; 1308 4th Avenue, San Francisco, CA 94122 (US). BANDMAN, Olga [US/US]; 366 Anna Avenue, Mountain View, CA 94043 (US). BAUGHN, Mariah, R. [US/US]; 14244 Santiago Road, San Leandro, CA 94577 (US). AZIMZAI, Yalda [US/US]; 2045 Rock Springs Drive, Hayward, CA 94545 (US). LU, Dyung, Aina, M. [US/US]; 55 Park Belmont Place, San Jose, CA 95136 (US). PATTERSON, Chandra [US/US]; 490 Sherwood Way #1, Menlo Park, CA 94025 (US).  <b>(74) Agents:</b> HAMLET-COX, Diana et al.; Incyte Genomics, Inc., 3160 Porter Drive, Palo Alto, CA 94304 (US).  <b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
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<b>(54) Title: EXTRACELLULAR SIGNALING MOLECULES</b>  <b>(57) Abstract</b> <p>The invention provides human extracellular signaling molecules (EXCS) and polynucleotides which identify and encode EXCS. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating or preventing disorders associated with expression of EXCS.</p>																														

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## EXTRACELLULAR SIGNALING MOLECULES

### TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of extracellular signaling  
5 molecules and to the use of these sequences in the diagnosis, treatment, and prevention of infections  
and gastrointestinal, neurological, reproductive, autoimmune/inflammatory, and cell proliferative  
disorders including cancer.

### BACKGROUND OF THE INVENTION

10 Protein transport and secretion are essential for cellular function. Protein transport is  
mediated by a signal peptide located at the amino terminus of the protein to be transported or  
secreted. The signal peptide is comprised of about ten to twenty hydrophobic amino acids which  
target the nascent protein from the ribosome to a particular membrane bound compartment such as the  
endoplasmic reticulum (ER). Proteins targeted to the ER may either proceed through the secretory  
15 pathway or remain in any of the secretory organelles such as the ER, Golgi apparatus, or lysosomes.  
Proteins that transit through the secretory pathway are either secreted into the extracellular space or  
retained in the plasma membrane. Secreted proteins are often synthesized as inactive precursors that  
are activated by post-translational processing events during transit through the secretory pathway.  
Such events include glycosylation, proteolysis, and removal of the signal peptide by a signal  
20 peptidase. Other events that may occur during protein transport include chaperone-dependent  
unfolding and folding of the nascent protein and interaction of the protein with a receptor or pore  
complex. Examples of secreted proteins with amino terminal signal peptides include receptors,  
extracellular matrix molecules, cytokines, hormones, growth and differentiation factors,  
neuropeptides, vasomediators, ion channels, transporters/pumps, and proteases. The discussion  
25 below focuses on the structure and function of cytokines, which play a key role in immune cell  
signaling. (Reviewed in Alberts, B. et al. (1994) Molecular Biology of The Cell, Garland Publishing,  
New York, NY, pp. 557-560, 582-592.)

Intercellular communication is essential for the growth and survival of multicellular  
organisms, and in particular, for the function of the endocrine, nervous, and immune systems. In  
30 addition, intercellular communication is critical for developmental processes such as tissue  
construction and organogenesis, in which cell proliferation, cell differentiation, and morphogenesis  
must be spatially and temporally regulated in a precise and coordinated manner. Cells communicate  
with one another through the secretion and uptake of diverse types of signaling molecules such as  
hormones, growth factors, neuropeptides, and cytokines.

35

Hormones

Hormones are signaling molecules that coordinately regulate basic physiological processes from embryogenesis throughout adulthood. These processes include metabolism, respiration, reproduction, excretion, fetal tissue differentiation and organogenesis, growth and development, homeostasis, and the stress response. Hormonal secretions and the nervous system are tightly integrated and interdependent. Hormones are secreted by endocrine glands, primarily the hypothalamus and pituitary, the thyroid and parathyroid, the pancreas, the adrenal glands, and the ovaries and testes.

The secretion of hormones into the circulation is tightly controlled. Hormones are often secreted in diurnal, pulsatile, and cyclic patterns. Hormone secretion is regulated by perturbations in blood biochemistry, by other upstream-acting hormones, by neural impulses, and by negative feedback loops. Blood hormone concentrations are constantly monitored and adjusted to maintain optimal, steady-state levels. Once secreted, hormones act only on those target cells that express specific receptors.

Most disorders of the endocrine system are caused by either hyposecretion or hypersecretion of hormones. Hyposecretion often occurs when a hormone's gland of origin is damaged or otherwise impaired. Hypersecretion often results from the proliferation of tumors derived from hormone-secreting cells. Inappropriate hormone levels may also be caused by defects in regulatory feedback loops or in the processing of hormone precursors. Endocrine malfunction may also occur when the target cell fails to respond to the hormone.

Hormones can be classified biochemically as polypeptides, steroids, eicosanoids, or amines. Polypeptides, which include diverse hormones such as insulin and growth hormone, vary in size and function and are often synthesized as inactive precursors that are processed intracellularly into mature, active forms. Amines, which include epinephrine and dopamine, are amino acid derivatives that function in neuroendocrine signaling. Steroids, which include the cholesterol-derived hormones estrogen and testosterone, function in sexual development and reproduction. Eicosanoids, which include prostaglandins and prostacyclins, are fatty acid derivatives that function in a variety of processes. Most polypeptides and some amines are soluble in the circulation where they are highly susceptible to proteolytic degradation within seconds after their secretion. Steroids and lipids are insoluble and must be transported in the circulation by carrier proteins. The following discussion will focus primarily on polypeptide hormones.

Hormones secreted by the hypothalamus and pituitary gland play a critical role in endocrine function by coordinately regulating hormonal secretions from other endocrine glands in response to neural signals. Hypothalamic hormones include thyrotropin-releasing hormone, gonadotropin-releasing hormone, somatostatin, growth-hormone releasing factor, corticotropin-releasing hormone.



substance P, dopamine, and prolactin-releasing hormone. These hormones directly regulate the secretion of hormones from the anterior lobe of the pituitary. Hormones secreted by the anterior pituitary include adrenocorticotrophic hormone (ACTH), melanocyte-stimulating hormone, somatotrophic hormones such as growth hormone and prolactin, glycoprotein hormones such as thyroid-stimulating hormone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH),  $\beta$ -lipotropin, and  $\beta$ -endorphins. These hormones regulate hormonal secretions from the thyroid, pancreas, and adrenal glands, and act directly on the reproductive organs to stimulate ovulation and spermatogenesis. The posterior pituitary synthesizes and secretes antidiuretic hormone (ADH, vasopressin) and oxytocin.

- 10 Disorders of the hypothalamus and pituitary often result from lesions such as primary brain tumors, adenomas, infarction associated with pregnancy, hypophysectomy, aneurysms, vascular malformations, thrombosis, infections, immunological disorders, and complications due to head trauma. Such disorders have profound effects on the function of other endocrine glands. Disorders associated with hypopituitarism include hypogonadism, Sheehan syndrome, diabetes insipidus,
- 15 Kallman's disease, Hand-Schuller-Christian disease, Letterer-Siwe disease, sarcoidosis, empty sella syndrome, and dwarfism. Disorders associated with hyperpituitarism include acromegaly, gigantism, and syndrome of inappropriate ADH secretion (SIADH), often caused by benign adenomas.

- Hormones secreted by the thyroid and parathyroid primarily control metabolic rates and the regulation of serum calcium levels, respectively. Thyroid hormones include calcitonin, somatostatin,
- 20 and thyroid hormone. The parathyroid secretes parathyroid hormone. Disorders associated with hypothyroidism include goiter, myxedema, acute thyroiditis associated with bacterial infection, subacute thyroiditis associated with viral infection, autoimmune thyroiditis (Hashimoto's disease), and cretinism. Disorders associated with hyperthyroidism include thyrotoxicosis and its various forms, Grave's disease, pretibial myxedema, toxic multinodular goiter, thyroid carcinoma, and
- 25 Plummer's disease. Disorders associated with hyperparathyroidism include Conn disease (chronic hypercalcemia) leading to bone resorption and parathyroid hyperplasia.

- Hormones secreted by the pancreas regulate blood glucose levels by modulating the rates of carbohydrate, fat, and protein metabolism. Pancreatic hormones include insulin, glucagon, amylin,  $\gamma$ -aminobutyric acid, gastrin, somatostatin, and pancreatic polypeptide. The principal disorder
- 30 associated with pancreatic dysfunction is diabetes mellitus caused by insufficient insulin activity. Diabetes mellitus is generally classified as either Type I (insulin-dependent, juvenile diabetes) or Type II (non-insulin-dependent, adult diabetes). The treatment of both forms by insulin replacement therapy is well known. Diabetes mellitus often leads to acute complications such as hypoglycemia (insulin shock), coma, diabetic ketoacidosis, lactic acidosis, and chronic complications leading to
- 35 disorders of the eye, kidney, skin, bone, joint, cardiovascular system, nervous system, and to

decreased resistance to infection.

The anatomy, physiology, and diseases related to hormonal function are reviewed in McCance, K. L. and Huether, S. E. (1994) Pathophysiology: The Biological Basis for Disease in Adults and Children. Mosby-Year Book, Inc., St. Louis, MO; Greenspan, F. S. and Baxter, J. D.

5 (1994) Basic and Clinical Endocrinology. Appleton and Lange, East Norwalk, CT.

#### Growth Factors

Growth factors are secreted proteins that mediate intercellular communication. Unlike hormones, which travel great distances via the circulatory system, most growth factors are primarily  
10 local mediators that act on neighboring cells. Most growth factors contain a hydrophobic N-terminal signal peptide sequence which directs the growth factor into the secretory pathway. Most growth factors also undergo post-translational modifications within the secretory pathway. These modifications can include proteolysis, glycosylation, phosphorylation, and intramolecular disulfide bond formation. Once secreted, growth factors bind to specific receptors on the surfaces of  
15 neighboring target cells, and the bound receptors trigger intracellular signal transduction pathways. These signal transduction pathways elicit specific cellular responses in the target cells. These responses can include the modulation of gene expression and the stimulation or inhibition of cell division, cell differentiation, and cell motility.

Growth factors fall into at least two broad and overlapping classes. The broadest class  
20 includes the large polypeptide growth factors, which are wide-ranging in their effects. These factors include epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth factor- $\beta$  (TGF- $\beta$ ), insulin-like growth factor (IGF), nerve growth factor (NGF), and platelet-derived growth factor (PDGF), each defining a family of numerous related factors. The large polypeptide growth factors, with the exception of NGF, act as mitogens on diverse cell types to stimulate wound healing,  
25 bone synthesis and remodeling, extracellular matrix synthesis, and proliferation of epithelial, epidermal, and connective tissues. Members of the TGF- $\beta$ , EGF, and FGF families also function as inductive signals in the differentiation of embryonic tissue. NGF functions specifically as a neurotrophic factor, promoting neuronal growth and differentiation.

EGF is a growth factor that stimulates proliferation of several epithelial tissues or cell lines.  
30 In addition to this mitogenic effect, EGF produces non-mitogenic effects in certain tissues. For example, in the stomach, EGF inhibits gastric acid secretion by parietal cells (Massagué, J. and Pandiella, A. (1993) *Annu. Rev. Biochem.* 62:515-541). EGF is produced as a larger precursor and contains an N-terminal signal peptide sequence that is thought to aid in localization of EGF to the plasma membrane. EGF contains three repeats of the calcium-binding EGF-like domain signature  
35 sequence. This signature sequence is about forty amino acid residues in length and includes six

conserved cysteine residues, and a calcium-binding site near the N-terminus of the signature sequence. A number of proteins that contain calcium-binding EGF-like domain signature sequences are involved in growth and differentiation. Examples include bone morphogenic protein 1, which induces the formation of cartilage and bone; crumbs, which is a Drosophila melanogaster epithelial development protein; Notch and a number of its homologs, which are involved in neural growth and differentiation; and transforming growth factor beta-1 binding protein (Expasy PROSITE document PDOC00913; Soler, C. and Carpenter, G., in Nicola, N.A. (1994) The Cytokine Facts Book, Oxford University Press, Oxford, UK, pp 193-197).

Another class of growth factors includes the hematopoietic growth factors, which are narrow in their target specificity. These factors stimulate the proliferation and differentiation of blood cells such as B-lymphocytes, T-lymphocytes, erythrocytes, platelets, eosinophils, basophils, neutrophils, macrophages, and their stem cell precursors. These factors include the colony-stimulating factors (G-CSF, M-CSF, GM-CSF, and CSF1-3), erythropoietin, and the cytokines. The cytokines are specialized hematopoietic factors secreted by cells of the immune system and are discussed in detail below.

Growth factors play critical roles in neoplastic transformation of cells in vitro and in tumor progression in vivo. Overexpression of the large polypeptide growth factors promotes the proliferation and transformation of cells in culture. Inappropriate expression of these growth factors by tumor cells in vivo may contribute to tumor vascularization and metastasis. Inappropriate activity of hematopoietic growth factors can result in anemias, leukemias, and lymphomas. Moreover, growth factors are both structurally and functionally related to oncoproteins, the potentially cancer-causing products of proto-oncogenes. Certain FGF and PDGF family members are themselves homologous to oncoproteins, whereas receptors for some members of the EGF, NGF, and FGF families are encoded by proto-oncogenes. Growth factors also affect the transcriptional regulation of both proto-oncogenes and oncosuppressor genes. (Pimentel, E. (1994) Handbook of Growth Factors, CRC Press, Ann Arbor, MI; McKay, I. and Leigh, I., eds. (1993) Growth Factors: A Practical Approach, Oxford University Press, New York, NY; Habenicht, A., ed. (1990) Growth Factors, Differentiation Factors, and Cytokines, Springer-Verlag, New York, NY.)

In addition, some of the large polypeptide growth factors play crucial roles in the induction of the primordial germ layers in the developing embryo. This induction ultimately results in the formation of the embryonic mesoderm, ectoderm, and endoderm which in turn provide the framework for the entire adult body plan. Disruption of this inductive process would be catastrophic to embryonic development.

Small Peptide Factors - Neuropeptides and Vasomediators

Neuropeptides and vasomediators (NP/VM) comprise a family of small peptide factors, typically of 20 amino acids or less. These factors generally function in neuronal excitation and inhibition of vasoconstriction/vasodilation, muscle contraction, and hormonal secretions from the brain and other endocrine tissues. Included in this family are neuropeptides and neuropeptide hormones such as bombesin, neuropeptide Y, neurotensin, neuromedin N, melanocortins, opioids, galanin, somatostatin, tachykinins, urotensin II and related peptides involved in smooth muscle stimulation, vasopressin, vasoactive intestinal peptide, and circulatory system-borne signaling molecules such as angiotensin, complement, calcitonin, endothelins, formyl-methionyl peptides, glucagon, cholecystikinin, gastrin, and many of the peptide hormones discussed above. NP/VMs can transduce signals directly, modulate the activity or release of other neurotransmitters and hormones, and act as catalytic enzymes in signaling cascades. The effects of NP/VMs range from extremely brief to long-lasting. (Reviewed in Martin, C. R. et al. (1985) Endocrine Physiology, Oxford University Press, New York, NY, pp. 57-62.)

The FMRFamide-like neuropeptides are a class of peptides found particularly in the brain, spinal cord, and gastrointestinal tract. FMRFamide-related peptides interact with opiate receptors (Raffa, R.B. (1991) NIDA Res. Monogr. 105:243-249).

Bombesin is a neuropeptide involved in appetite and stress response. Bombesin-like peptides are released at the central nucleus of the amygdala in response to both stress and food intake (Merali, Z. et al. (1998) J. Neurosci. 18:4758-4766). Bombesin has been shown to decrease food intake, increase the duration of slow wave sleep, and increase the concentration of both blood glucose and glucagon (Even, P.C. et al. (1991) Physiol. Behav. 49:439-442).

Cytokines

Cytokines comprise a family of signaling molecules that modulate the immune system and the inflammatory response. Cytokines are usually secreted by leukocytes, or white blood cells, in response to injury or infection. Cytokines function as growth and differentiation factors that act primarily on cells of the immune system such as B- and T-lymphocytes, monocytes, macrophages, and granulocytes. Like other signaling molecules, cytokines bind to specific plasma membrane receptors and trigger intracellular signal transduction pathways which alter gene expression patterns. There is considerable potential for the use of cytokines in the treatment of inflammation and immune system disorders.

Cytokine structure and function have been extensively characterized in vitro. Most cytokines are small polypeptides of about 30 kilodaltons or less. Over 50 cytokines have been identified from human and rodent sources. Examples of cytokine subfamilies include the interferons (IFN- $\alpha$ , - $\beta$ , and - $\gamma$ ), the interleukins (IL1-IL13), the tumor necrosis factors (TNF- $\alpha$  and - $\beta$ ), and the chemokines.

Many cytokines have been produced using recombinant DNA techniques, and the activities of individual cytokines have been determined in vitro. These activities include regulation of leukocyte proliferation, differentiation, and motility.

The activity of an individual cytokine in vitro may not reflect the full scope of that cytokine's activity in vivo. Cytokines are not expressed individually in vivo but are instead expressed in combination with a multitude of other cytokines when the organism is challenged with a stimulus. Together, these cytokines collectively modulate the immune response in a manner appropriate for that particular stimulus. Therefore, the physiological activity of a cytokine is determined by the stimulus itself and by complex interactive networks among co-expressed cytokines which may demonstrate both synergistic and antagonistic relationships.

Recently, a unique cytokine has been characterized with a likely role in regulating fibrogenesis associated with cases of chronic inflammation. This cytokine, fibrosin, has no obvious homology with other proteins in the GenBank database. A 36-amino acid synthetic peptide constructed from the deduced amino acid sequence of human fibrosin stimulates fibroblast growth at subnanomolar concentrations. Tissue fibrosis is a serious complication that accompanies chronic inflammation. A number of fibrogenic cytokines act in concert to stimulate the growth of fibroblasts and the extracellular matrix components associated with fibrosis. (Prakash, S. and P.W. Robbins (1998) DNA Cell Bio. 17:879-884).

Interleukin-10 (IL-10) is one of the better studied cytokines. In humans IL-10 is a secreted 18 kilodalton protein produced by some T and B lymphocytes and macrophages. There are four cysteine residues in the IL-10 protein that are conserved in human, murine and viral IL-10. Two of these cysteines are involved in the formation of intramolecular disulfide bonds. IL-10 can inhibit cytokine production by T cells, inhibit cytokine synthesis by macrophages, and stimulate proliferation of thymocytes, T cells and B cells in addition to megakaryocytes, and other haemopoietic cells. (Nicola, N.A. (1994) Guidebook to Cytokines and Their Receptors Oxford University Press, New York, NY, pp. 84-85).

Low homologies between various cytokine family members make it difficult to establish relationships between known members and newly discovered cytokines. Homologies within families can be 25% or lower, and conserved amino acids may be clustered in small domains or repeats. Often only a seeming chance similarity exists between family members until structural information clarifies homologies. Conserved disulfide bridges are a strong indicator of conserved or similar protein structure and or folding. For example, IL-10 molecules from several sources share four conserved cysteines that participate in structure determining intramolecular contacts. (Callard, R. and A. Gearing. (1994) In The Cytokine Factsbook. Academic Press, San Diego CA. p. 18).

Chemokines comprise a cytokine subfamily with over 30 members. (Reviewed in Wells, T.

N. C. and Peitsch, M. C. (1997) *J. Leukoc. Biol.* 61:545-550.) Chemokines were initially identified as chemotactic proteins that recruit monocytes and macrophages to sites of inflammation. Recent evidence indicates that chemokines may also play key roles in hematopoiesis and HIV-1 infection. Chemokines are small proteins which range from about 6-15 kilodaltons in molecular weight.

- 5 Chemokines are further classified as C, CC, CXC, or CX<sub>3</sub>C based on the number and position of critical cysteine residues. The CC chemokines, for example, each contain a conserved motif consisting of two consecutive cysteines followed by two additional cysteines which occur downstream at 24- and 16-residue intervals, respectively (ExPASy PROSITE database, documents PS00472 and PDOC00434). The presence and spacing of these four cysteine residues are highly
- 10 conserved, whereas the intervening residues diverge significantly. However, a conserved tyrosine located about 15 residues downstream of the cysteine doublet seems to be important for chemotactic activity. Most of the human genes encoding CC chemokines are clustered on chromosome 17, although there are a few examples of CC chemokine genes that map elsewhere. Other chemokines include lymphotactin (C chemokine); macrophage chemotactic and activating factor (MCAF/MCP-1; CC chemokine); platelet factor 4 and IL-8 (CXC chemokines); and fractalkine and neurotractin
- 15 (CX<sub>3</sub>C chemokines). (Reviewed in Luster, A. D. (1998) *N. Engl. J. Med.* 338:436-445.)

- The discovery of new extracellular signaling molecules and the polynucleotides encoding them satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention, and treatment of infections and gastrointestinal, neurological, reproductive,
- 20 autoimmune/inflammatory, and cell proliferative disorders including cancer.

### SUMMARY OF THE INVENTION

- The invention features purified polypeptides, extracellular signaling molecules, referred to collectively as "EXCS" and individually as "EXCS-1," "EXCS-2," "EXCS-3," "EXCS-4," "EXCS-5," "EXCS-6," "EXCS-7," "EXCS-8," "EXCS-9," "EXCS-10," "EXCS-11," "EXCS-12," "EXCS-13," "EXCS-14," "EXCS-15," "EXCS-16," "EXCS-17," "EXCS-18," "EXCS-19," "EXCS-20," "EXCS-21," "EXCS-22," "EXCS-23," "EXCS-24," "EXCS-25," and "EXCS-26." In one aspect, the invention provides an isolated polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, b) a naturally occurring amino acid sequence having at least
- 30 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-26. In one alternative, the invention provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:1-26.

- 35 The invention further provides an isolated polynucleotide encoding a polypeptide comprising

- a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-26. b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-26. c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-26. or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-26. In one alternative, the polynucleotide is selected from the group consisting of SEQ ID NO:27-52.

Additionally, the invention provides a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-26. c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-26. or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-26. In one alternative, the invention provides a cell transformed with the recombinant polynucleotide. In another alternative, the invention provides a transgenic organism comprising the recombinant polynucleotide.

The invention also provides a method for producing a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-26. The method comprises a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding the polypeptide, and b) recovering the polypeptide so expressed.

Additionally, the invention provides an isolated antibody which specifically binds to a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-26.

The invention further provides an isolated polynucleotide comprising a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:27-52. b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:27-52, c) a polynucleotide sequence complementary to a).

or d) a polynucleotide sequence complementary to b). In one alternative, the polynucleotide comprises at least 60 contiguous nucleotides.

Additionally, the invention provides a method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide comprising a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:27-52, b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:27-52, c) a polynucleotide sequence complementary to a), or d) a polynucleotide sequence complementary to b). The method comprises a) hybridizing the sample with a probe comprising at least 16 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide, and b) detecting the presence or absence of said hybridization complex, and optionally, if present, the amount thereof. In one alternative, the probe comprises at least 30 contiguous nucleotides. In another alternative, the probe comprises at least 60 contiguous nucleotides.

The invention further provides a pharmaceutical composition comprising an effective amount of a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, and a pharmaceutically acceptable excipient. The invention additionally provides a method of treating a disease or condition associated with decreased expression of functional EXCS, comprising administering to a patient in need of such treatment the pharmaceutical composition.

The invention also provides a method for screening a compound for effectiveness as an agonist of a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-26. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting agonist activity in the sample. In one alternative, the invention provides a pharmaceutical composition comprising an agonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with decreased expression of functional EXCS, comprising



administering to a patient in need of such treatment the pharmaceutical composition.

Additionally, the invention provides a method for screening a compound for effectiveness as an antagonist of a polypeptide comprising a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, or d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-26. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting antagonist activity in the sample. In one alternative, the invention provides a pharmaceutical composition comprising an antagonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with overexpression of functional EXCS, comprising administering to a patient in need of such treatment the pharmaceutical composition.

The invention further provides a method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO:27-52, the method comprising a) exposing a sample comprising the target polynucleotide to a compound, and b) detecting altered expression of the target polynucleotide.

#### BRIEF DESCRIPTION OF THE TABLES AND FIGURE

Table 1 shows polypeptide and nucleotide sequence identification numbers (SEQ ID NOs), clone identification numbers (clone IDs), cDNA libraries, and cDNA fragments used to assemble full-length sequences encoding EXCS.

Table 2 shows features of each polypeptide sequence, including potential motifs, homologous sequences, and methods, algorithms, and searchable databases used for analysis of EXCS.

Table 3 shows selected fragments of each nucleic acid sequence; the tissue-specific expression patterns of each nucleic acid sequence as determined by northern analysis; diseases, disorders, or conditions associated with these tissues; and the vector into which each cDNA was cloned.

Table 4 describes the tissues used to construct the cDNA libraries from which cDNA clones encoding EXCS were isolated.

Table 5 shows the tools, programs, and algorithms used to analyze EXCS, along with applicable descriptions, references, and threshold parameters.

Figures 1A and 1B show the amino acid sequence alignment among EXCS-18 (SEQ ID

NO:18), interleukin-10 (GI 511295), IL-10 precursor (GI 1841298) and interleukin-10 precursor-human (GI 106805), produced using the multisequence alignment program of LASERGENE software (DNASTAR, Madison WI).

## DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular machines, materials and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any machines, materials, and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

## DEFINITIONS

"EXCS" refers to the amino acid sequences of substantially purified EXCS obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and human, and from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist" refers to a molecule which intensifies or mimics the biological activity of EXCS. Agonists may include proteins, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of EXCS either by directly interacting with EXCS or by acting on components of the biological pathway in which EXCS participates.

An "allelic variant" is an alternative form of the gene encoding EXCS. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. A gene may have none, one, or many allelic variants of its naturally occurring form. Common mutational changes which give rise to

allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides.

Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

“Altered” nucleic acid sequences encoding EXCS include those sequences with deletions, 5 insertions, or substitutions of different nucleotides, resulting in a polypeptide the same as EXCS or a polypeptide with at least one functional characteristic of EXCS. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding EXCS, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding 10 EXCS. The encoded protein may also be “altered,” and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent EXCS. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of EXCS is retained. For example, 15 negatively charged amino acids may include aspartic acid and glutamic acid, and positively charged amino acids may include lysine and arginine. Amino acids with uncharged polar side chains having similar hydrophilicity values may include: asparagine and glutamine; and serine and threonine. Amino acids with uncharged side chains having similar hydrophilicity values may include: leucine, isoleucine, and valine; glycine and alanine; and phenylalanine and tyrosine.

20 The terms “amino acid” and “amino acid sequence” refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. Where “amino acid sequence” is recited to refer to an amino acid sequence of a naturally occurring protein molecule, “amino acid sequence” and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein 25 molecule.

“Amplification” relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

The term “antagonist” refers to a molecule which inhibits or attenuates the biological activity 30 of EXCS. Antagonists may include proteins such as antibodies, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of EXCS either by directly interacting with EXCS or by acting on components of the biological pathway in which EXCS participates.

The term “antibody” refers to intact immunoglobulin molecules as well as to fragments 35 thereof, such as Fab, F(ab')<sub>2</sub>, and Fv fragments, which are capable of binding an epitopic determinant.

Antibodies that bind EXCS polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly  
5 used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant" refers to that region of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies  
10 which bind specifically to antigenic determinants (particular regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense" refers to any composition capable of base-pairing with the "sense" strand of a specific nucleic acid sequence. Antisense compositions may include DNA; RNA; peptide  
15 nucleic acid (PNA); oligonucleotides having modified backbone linkages such as phosphorothioates, methylphosphonates, or benzylphosphonates; oligonucleotides having modified sugar groups such as 2'-methoxyethyl sugars or 2'-methoxyethoxy sugars; or oligonucleotides having modified bases such as 5-methyl cytosine, 2'-deoxyuracil, or 7-deaza-2'-deoxyguanosine. Antisense molecules may be produced by any method including chemical synthesis or transcription. Once introduced into a cell,  
20 the complementary antisense molecule base-pairs with a naturally occurring nucleic acid sequence produced by the cell to form duplexes which block either transcription or translation. The designation "negative" or "minus" can refer to the antisense strand, and the designation "positive" or "plus" can refer to the sense strand of a reference DNA molecule.

The term "biologically active" refers to a protein having structural, regulatory, or biochemical  
25 functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic EXCS, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The terms "complementary" and "complementarity" refer to the natural binding of polynucleotides by base pairing. For example, the sequence "5' A-G-T 3'" bonds to the  
30 complementary sequence "3' T-C-A 5'." Complementarity between two single-stranded molecules may be "partial," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which  
35 depend upon binding between nucleic acid strands, and in the design and use of peptide nucleic acid

A "composition comprising a given polynucleotide sequence" and a "composition comprising a given amino acid sequence" refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution.

5 Compositions comprising polynucleotide sequences encoding EXCS or fragments of EXCS may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate: SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

10 "Consensus sequence" refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, extended using the XL-PCR kit (Perkin-Elmer, Norwalk CT) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from the overlapping sequences of one or more Incyte Clones and, in some cases, one or more public domain ESTs, using a computer program for fragment assembly, such as the GELVIEW fragment assembly system (GCG, Madison  
15 WI). Some sequences have been both extended and assembled to produce the consensus sequence.

"Conservative amino acid substitutions" are those substitutions that, when made, least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids which may be substituted for an original amino acid in a protein and which are regarded  
20 as conservative amino acid substitutions.

	Original Residue	Conservative Substitution
	Ala	Gly, Ser
	Arg	His, Lys
	Asn	Asp, Gln, His
25	Asp	Asn, Glu
	Cys	Ala, Ser
	Gln	Asn, Glu, His
	Glu	Asp, Gln, His
	Gly	Ala
30	His	Asn, Arg, Gln, Glu
	Ile	Leu, Val
	Leu	Ile, Val
	Lys	Arg, Gln, Glu
	Met	Leu, Ile
35	Phe	His, Met, Leu, Trp, Tyr
	Ser	Cys, Thr
	Thr	Ser, Val
	Trp	Phe, Tyr
	Tyr	His, Phe, Trp
40	Val	Ile, Leu, Thr

Conservative amino acid substitutions generally maintain (a) the structure of the polypeptide

backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation.

(b) the charge or hydrophobicity of the molecule at the site of the substitution, and/or (c) the bulk of the side chain.

A "deletion" refers to a change in the amino acid or nucleotide sequence that results in the  
5 absence of one or more amino acid residues or nucleotides.

The term "derivative" refers to the chemical modification of a polypeptide sequence, or a polynucleotide sequence. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, hydroxyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function  
10 of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

A "fragment" is a unique portion of EXCS or the polynucleotide encoding EXCS which is identical in sequence to but shorter in length than the parent sequence. A fragment may comprise up  
15 to the entire length of the defined sequence, minus one nucleotide/amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous nucleotides or amino acid residues. A fragment used as a probe, primer, antigen, therapeutic molecule, or for other purposes, may be at least 5, 10, 15, 16, 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous nucleotides or amino acid residues in length. Fragments may be preferentially selected from certain regions of a molecule. For  
20 example, a polypeptide fragment may comprise a certain length of contiguous amino acids selected from the first 250 or 500 amino acids (or first 25% or 50% of a polypeptide) as shown in a certain defined sequence. Clearly these lengths are exemplary, and any length that is supported by the specification, including the Sequence Listing, tables, and figures, may be encompassed by the present embodiments.

25 A fragment of SEQ ID NO:27-52 comprises a region of unique polynucleotide sequence that specifically identifies SEQ ID NO:27-52, for example, as distinct from any other sequence in the same genome. A fragment of SEQ ID NO:27-52 is useful, for example, in hybridization and amplification technologies and in analogous methods that distinguish SEQ ID NO:27-52 from related polynucleotide sequences. The precise length of a fragment of SEQ ID NO:27-52 and the region of  
30 SEQ ID NO:27-52 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A fragment of SEQ ID NO:1-26 is encoded by a fragment of SEQ ID NO:27-52. A fragment of SEQ ID NO:1-26 comprises a region of unique amino acid sequence that specifically identifies SEQ ID NO:1-26. For example, a fragment of SEQ ID NO:1-26 is useful as an immunogenic peptide  
35 for the development of antibodies that specifically recognize SEQ ID NO:1-26. The precise length of

a fragment of SEQ ID NO:1-26 and the region of SEQ ID NO:1-26 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

The term "similarity" refers to a degree of complementarity. There may be partial similarity or complete similarity. The word "identity" may substitute for the word "similarity." A partially complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred to as "substantially similar." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or northern blot, solution hybridization, and the like) under conditions of reduced stringency. A substantially similar sequence or hybridization probe will compete for and inhibit the binding of a completely similar (identical) sequence to the target sequence under conditions of reduced stringency. This is not to say that conditions of reduced stringency are such that non-specific binding is permitted, as reduced stringency conditions require that the binding of two sequences to one another be a specific (i.e., a selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% similarity or identity). In the absence of non-specific binding, the substantially similar sequence or probe will not hybridize to the second non-complementary target sequence.

The phrases "percent identity" and "% identity," as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences.

Percent identity between polynucleotide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program. This program is part of the LASERGENE software package, a suite of molecular biological analysis programs (DNASTAR, Madison WI). CLUSTAL V is described in Higgins, D.G. and P.M. Sharp (1989) CABIOS 5:151-153 and in Higgins, D.G. et al. (1992) CABIOS 8:189-191. For pairwise alignments of polynucleotide sequences, the default parameters are set as follows: Ktuple=2, gap penalty=5, window=4, and "diagonals saved"=4. The "weighted" residue weight table is selected as the default. Percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polynucleotide sequence pairs.

Alternatively, a suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410), which is available

from several sources, including the NCBI, Bethesda, MD, and on the Internet at

<http://www.ncbi.nlm.nih.gov/BLAST/>. The BLAST software suite includes various sequence analysis programs including "blastn," that is used to align a known polynucleotide sequence with other polynucleotide sequences from a variety of databases. Also available is a tool called "BLAST 2

- 5 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at <http://www.ncbi.nlm.nih.gov/gorf/bl2.html>. The "BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed below). BLAST programs are commonly used with gap and other parameters set to default settings. For example, to compare two nucleotide sequences, one may use blastn with the "BLAST 2 Sequences" tool Version
- 10 2.0.9 (May-07-1999) set at default parameters. Such default parameters may be, for example:

*Matrix: BLOSUM62*

*Reward for match: 1*

*Penalty for mismatch: -2*

*Open Gap: 5 and Extension Gap: 2 penalties*

- 15 *Gap x drop-off: 50*

*Expect: 10*

*Word Size: 11*

*Filter: on*

- Percent identity may be measured over the length of an entire defined sequence, for example,
- 20 as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures, or Sequence Listing, may be used to
- 25 describe a length over which percentage identity may be measured.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein.

- 30 The phrases "percent identity" and "% identity," as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well-known. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the hydrophobicity and acidity at the
- 35 site of substitution, thus preserving the structure (and therefore function) of the polypeptide.



Percent identity between polypeptide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program (described and referenced above). For pairwise alignments of polypeptide sequences using CLUSTAL V, the default parameters are set as follows: Ktuple=1, gap  
5 penalty=3, window=5, and "diagonals saved"=5. The PAM250 matrix is selected as the default residue weight table. As with polynucleotide alignments, the percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polypeptide sequence pairs.

Alternatively the NCBI BLAST software suite may be used. For example, for a pairwise comparison of two polypeptide sequences, one may use the "BLAST 2 Sequences" tool Version 2.0.9  
10 (May-07-1999) with blastp set at default parameters. Such default parameters may be, for example:

*Matrix: BLOSUM62*

*Open Gap: 11 and Extension Gap: 1 penalties*

*Gap x drop-off: 50*

*Expect: 10*

15 *Word Size: 3*

*Filter: on*

Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for  
20 instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

"Human artificial chromosomes" (HACs) are linear microchromosomes which may contain  
25 DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance.

The term "humanized antibody" refers to antibody molecules in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

30 "Hybridization" refers to the process by which a polynucleotide strand anneals with a complementary strand through base pairing under defined hybridization conditions. Specific hybridization is an indication that two nucleic acid sequences share a high degree of identity. Specific hybridization complexes form under permissive annealing conditions and remain hybridized after the "washing" step(s). The washing step(s) is particularly important in determining the  
35 stringency of the hybridization process, with more stringent conditions allowing less non-specific

binding, i.e., binding between pairs of nucleic acid strands that are not perfectly matched. Permissive conditions for annealing of nucleic acid sequences are routinely determinable by one of ordinary skill in the art and may be consistent among hybridization experiments, whereas wash conditions may be varied among experiments to achieve the desired stringency, and therefore hybridization specificity.

- 5 Permissive annealing conditions occur, for example, at 68°C in the presence of about 6 x SSC, about 1% (w/v) SDS, and about 100 µg/ml denatured salmon sperm DNA.

Generally, stringency of hybridization is expressed, in part, with reference to the temperature under which the wash step is carried out. Generally, such wash temperatures are selected to be about 5°C to 20°C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. An equation for calculating  $T_m$  and conditions for nucleic acid hybridization are well known and can be found in Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; specifically see volume 2, chapter 9.

- 15 High stringency conditions for hybridization between polynucleotides of the present invention include wash conditions of 68°C in the presence of about 0.2 x SSC and about 0.1% SDS, for 1 hour. Alternatively, temperatures of about 65°C, 60°C, 55°C, or 42°C may be used. SSC concentration may be varied from about 0.1 to 2 x SSC, with SDS being present at about 0.1%. Typically, blocking reagents are used to block non-specific hybridization. Such blocking reagents include, for instance, denatured salmon sperm DNA at about 100-200 µg/ml. Organic solvent, such as formamide at a concentration of about 35-50% v/v, may also be used under particular circumstances, such as for RNA:DNA hybridizations. Useful variations on these wash conditions will be readily apparent to those of ordinary skill in the art. Hybridization, particularly under high stringency conditions, may be suggestive of evolutionary similarity between the nucleotides. Such similarity is strongly indicative of a similar role for the nucleotides and their encoded polypeptides.

25 The term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g.,  $C_0t$  or  $R_0t$  analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words "insertion" and "addition" refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively.

- 35 "Immune response" can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression

of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

An "immunogenic fragment" is a polypeptide or oligopeptide fragment of EXCS which is capable of eliciting an immune response when introduced into a living organism, for example, a mammal. The term "immunogenic fragment" also includes any polypeptide or oligopeptide fragment of EXCS which is useful in any of the antibody production methods disclosed herein or known in the art.

The term "microarray" refers to an arrangement of distinct polynucleotides on a substrate.

The terms "element" and "array element" in a microarray context, refer to hybridizable polynucleotides arranged on the surface of a substrate.

The term "modulate" refers to a change in the activity of EXCS. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of EXCS.

The phrases "nucleic acid" and "nucleic acid sequence" refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material.

"Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

"Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

"Probe" refers to nucleic acid sequences encoding EXCS, their complements, or fragments thereof, which are used to detect identical, allelic or related nucleic acid sequences. Probes are isolated oligonucleotides or polynucleotides attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes.

"Primers" are short nucleic acids, usually DNA oligonucleotides, which may be annealed to a target polynucleotide by complementary base-pairing. The primer may then be extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification (and identification) of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR).

Probes and primers as used in the present invention typically comprise at least 15 contiguous nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or at least 150 consecutive nucleotides of the disclosed nucleic acid sequences. Probes and primers  
5 may be considerably longer than these examples, and it is understood that any length supported by the specification, including the tables, figures, and Sequence Listing, may be used.

Methods for preparing and using probes and primers are described in the references, for example Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; Ausubel et al., 1987, Current Protocols in Molecular Biology,  
10 Greene Publ. Assoc. & Wiley-Intersciences, New York NY; Innis et al., 1990, PCR Protocols. A Guide to Methods and Applications, Academic Press, San Diego CA. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge MA).

Oligonucleotides for use as primers are selected using software known in the art for such  
15 purpose. For example, OLIGO 4.06 software is useful for the selection of PCR primer pairs of up to 100 nucleotides each, and for the analysis of oligonucleotides and larger polynucleotides of up to 5,000 nucleotides from an input polynucleotide sequence of up to 32 kilobases. Similar primer selection programs have incorporated additional features for expanded capabilities. For example, the PrimOU primer selection program (available to the public from the Genome Center at University of  
20 Texas South West Medical Center, Dallas TX) is capable of choosing specific primers from megabase sequences and is thus useful for designing primers on a genome-wide scope. The Primer3 primer selection program (available to the public from the Whitehead Institute/MIT Center for Genome Research, Cambridge MA) allows the user to input a "mispriming library," in which sequences to avoid as primer binding sites are user-specified. Primer3 is useful, in particular, for the  
25 selection of oligonucleotides for microarrays. (The source code for the latter two primer selection programs may also be obtained from their respective sources and modified to meet the user's specific needs.) The PrimeGen program (available to the public from the UK Human Genome Mapping Project Resource Centre, Cambridge UK) designs primers based on multiple sequence alignments, thereby allowing selection of primers that hybridize to either the most conserved or least conserved  
30 regions of aligned nucleic acid sequences. Hence, this program is useful for identification of both unique and conserved oligonucleotides and polynucleotide fragments. The oligonucleotides and polynucleotide fragments identified by any of the above selection methods are useful in hybridization technologies, for example, as PCR or sequencing primers, microarray elements, or specific probes to identify fully or partially complementary polynucleotides in a sample of nucleic acids. Methods of  
35 oligonucleotide selection are not limited to those described above.

A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques such as those described in Sambrook, supra. The term recombinant includes nucleic acids that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence. Such a recombinant nucleic acid may be part of a vector that is used, for example, to transform a cell.

Alternatively, such recombinant nucleic acids may be part of a viral vector, e.g., based on a vaccinia virus, that could be used to vaccinate a mammal wherein the recombinant nucleic acid is expressed, inducing a protective immunological response in the mammal.

An "RNA equivalent," in reference to a DNA sequence, is composed of the same linear sequence of nucleotides as the reference DNA sequence with the exception that all occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

The term "sample" is used in its broadest sense. A sample suspected of containing nucleic acids encoding EXCS, or fragments thereof, or EXCS itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

The terms "specific binding" and "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, an antagonist, a small molecule, or any natural or synthetic binding composition. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

The term "substantially purified" refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least 60% free, preferably at least 75% free, and most preferably at least 90% free from other components with which they are naturally associated.

A "substitution" refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

"Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers,

microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

"Transformation" describes a process by which exogenous DNA enters and changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "transgenic organism," as used herein, is any organism, including but not limited to animals and plants, in which one or more of the cells of the organism contains heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or in vitro fertilization, but rather is directed to the introduction of a recombinant DNA molecule. The transgenic organisms contemplated in accordance with the present invention include bacteria, cyanobacteria, fungi, and plants and animals. The isolated DNA of the present invention can be introduced into the host by methods known in the art, for example infection, transfection, transformation or transconjugation. Techniques for transferring the DNA of the present invention into such organisms are widely known and provided in references such as Sambrook et al. (1989), supra.

A "variant" of a particular nucleic acid sequence is defined as a nucleic acid sequence having at least 40% sequence identity to the particular nucleic acid sequence over a certain length of one of the nucleic acid sequences using blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of nucleic acids may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or greater sequence identity over a certain defined length. A variant may be described as, for example, an "allelic" (as defined above), "splice," "species," or "polymorphic" variant. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or lack domains that are present in the reference molecule. Species variants are polynucleotide sequences that vary from one species to

another. The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one nucleotide base. The  
 5 presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

A "variant" of a particular polypeptide sequence is defined as a polypeptide sequence having at least 40% sequence identity to the particular polypeptide sequence over a certain length of one of the polypeptide sequences using blastp with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-  
 10 1999) set at default parameters. Such a pair of polypeptides may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% or greater sequence identity over a certain defined length of one of the polypeptides.

### THE INVENTION

The invention is based on the discovery of new human extracellular signaling molecules  
 15 (EXCS), the polynucleotides encoding EXCS, and the use of these compositions for the diagnosis, treatment, or prevention of infections and gastrointestinal, neurological, reproductive, autoimmune/inflammatory, and cell proliferative disorders including cancer.

Table 1 lists the Incyte clones used to assemble full length nucleotide sequences encoding EXCS. Columns 1 and 2 show the sequence identification numbers (SEQ ID NOs) of the polypeptide  
 20 and nucleotide sequences, respectively. Column 3 shows the clone IDs of the Incyte clones in which nucleic acids encoding each EXCS were identified, and column 4 shows the cDNA libraries from which these clones were isolated. Column 5 shows Incyte clones and their corresponding cDNA libraries. Clones for which cDNA libraries are not indicated were derived from pooled cDNA libraries. In some cases, GenBank sequence identifiers are also shown in column 5. The Incyte clones  
 25 and GenBank cDNA sequences, where indicated, in column 5 were used to assemble the consensus nucleotide sequence of each EXCS and are useful as fragments in hybridization technologies.

The columns of Table 2 show various properties of each of the polypeptides of the invention: column 1 references the SEQ ID NO; column 2 shows the number of amino acid residues in each polypeptide; column 3 shows potential phosphorylation sites; column 4 shows potential glycosylation  
 30 sites; column 5 shows the amino acid residues comprising signature sequences and motifs; column 6 shows homologous sequences as identified by BLAST analysis along with relevant citations, all of which are expressly incorporated by reference herein in their entirety; and column 7 shows analytical methods and in some cases, searchable databases to which the analytical methods were applied. The methods of column 7 were used to characterize each polypeptide through sequence homology and  
 35 protein motifs. Of particular note is the presence of one or more cysteine residues in each of the

polypeptide sequences of SEQ ID NO:1-10.

Figures 1A. and 1B show the amino acid sequence alignment among EXCS-18 (SEQ ID NO:18), interleukin-10 (GI 511295; SEQ ID NO:53), interleukin-10 precursor (GI 1841298; SEQ ID NO:54) and interleukin-10 precursor-human (GI 106805; SEQ ID NO:55) with conserved amino acid residues boxed. The alignments illustrate an overall protein length in the range of 178-179 residues for all four proteins, indicating that SEQ ID NO:18 shares structural similarity with GI 511295, GI 1841298, and GI 106805 on the basis of molecule length. It is also noteworthy that SEQ ID NO:18 shares four out of six highly conserved cysteine residues found in GI 511295, GI 1841298, and GI 106805 at positions C20, C40, C89 and C132. Furthermore, three of these cysteines (C40, C89 and C132) are known to be directly involved in intramolecular disulfide bridge formation within IL-10 molecules, thus illustrating homology and possible secondary structural similarity of SEQ ID NO:18 to GI 511295, GI 1841298, and GI 106805. Additional homology of SEQ ID NO:18 to GI 511295, GI 1841298, and GI 106805 is apparent as numerous conserved amino acid residues, including a number of basic and acidic residues, and in particular, two structurally relevant proline residues at positions 106 and 113.

The columns of Table 3 show the tissue-specificity and diseases, disorders, or conditions associated with nucleotide sequences encoding EXCS. The first column of Table 3 lists the nucleotide SEQ ID NOs. Column 2 lists fragments of the nucleotide sequences of column 1. These fragments are useful, for example, in hybridization or amplification technologies to identify SEQ ID NO:27-52 and to distinguish between SEQ ID NO:27-52 and related polynucleotide sequences. The polypeptides encoded by these fragments are useful, for example, as immunogenic peptides. Column 3 lists tissue categories which express EXCS as a fraction of total tissues expressing EXCS. Column 4 lists diseases, disorders, or conditions associated with those tissues expressing EXCS as a fraction of total tissues expressing EXCS. Of particular note is the expression of SEQ ID NO:30. This sequence is detected in six cDNA libraries, all of which were constructed independently using RNA isolated from prostate tissue. Therefore, SEQ ID NO:30 is useful, for example, as a prostate-specific marker for tissue-typing and for diagnosis of diseases of the prostate. SEQ ID NO:43 is specifically expressed in islet cells and in islet cell tumor only. Of particular note is the expression of SEQ ID NO:45 exclusively in hematopoietic/immune tissues. Column 5 lists the vectors used to subclone each cDNA library.

The columns of Table 4 show descriptions of the tissues used to construct the cDNA libraries from which cDNA clones encoding EXCS were isolated. Column 1 references the nucleotide SEQ ID NOs, column 2 shows the cDNA libraries from which these clones were isolated, and column 3 shows the tissue origins and other descriptive information relevant to the cDNA libraries in column 2.

SEQ ID NO:47 maps to chromosome 2 within the interval from 77.1 to 84.0 centiMorgans.



This interval also contains a gene associated with stimulation of DNA synthesis.

The invention also encompasses EXCS variants. A preferred EXCS variant is one which has at least about 80%, or alternatively at least about 90%, or even at least about 95% amino acid sequence identity to the EXCS amino acid sequence, and which contains at least one functional or structural characteristic of EXCS.

The invention also encompasses polynucleotides which encode EXCS. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:27-52, which encodes EXCS. The polynucleotide sequences of SEQ ID NO:27-52, as presented in the Sequence Listing, embrace the equivalent RNA sequences, wherein occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

The invention also encompasses a variant of a polynucleotide sequence encoding EXCS. In particular, such a variant polynucleotide sequence will have at least about 70%, or alternatively at least about 85%, or even at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding EXCS. A particular aspect of the invention encompasses a variant of a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:27-52 which has at least about 70%, or alternatively at least about 85%, or even at least about 95% polynucleotide sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:27-52. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of EXCS.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding EXCS, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring EXCS, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode EXCS and its variants are generally capable of hybridizing to the nucleotide sequence of the naturally occurring EXCS under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding EXCS or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide

sequence encoding EXCS and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode EXCS and EXCS derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding EXCS or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:27-52 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) *Methods Enzymol.* 152:399-407; Kimmel, A.R. (1987) *Methods Enzymol.* 152:507-511.) Hybridization conditions, including annealing and wash conditions, are described in "Definitions."

Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (Perkin-Elmer), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the MICROLAB 2200 liquid transfer system (Hamilton, Reno NV), PTC200 thermal cycler (MJ Research, Watertown MA) and ABI CATALYST 800 thermal cycler (Perkin-Elmer). Sequencing is then carried out using either the ABI 373 or 377 DNA sequencing system (Perkin-Elmer), the MEGABACE 1000 DNA sequencing system (Molecular Dynamics, Sunnyvale CA), or other systems known in the art. The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short Protocols in Molecular Biology, John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853.)

The nucleic acid sequences encoding EXCS may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) *PCR Methods Applic.* 2:318-322.) Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising

a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) *Nucleic Acids Res.* 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) *PCR Methods Applic.* 1:111-119.) In this method, multiple restriction enzyme  
5 digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) *Nucleic Acids Res.* 19:3055-3060). Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto CA) to walk genomic DNA. This procedure avoids the need to screen libraries  
10 and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences, Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

15 When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

20 Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate  
25 software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, Perkin-Elmer), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof  
30 which encode EXCS may be cloned in recombinant DNA molecules that direct expression of EXCS, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express EXCS.

The nucleotide sequences of the present invention can be engineered using methods generally  
35 known in the art in order to alter EXCS-encoding sequences for a variety of purposes including, but

not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

The nucleotides of the present invention may be subjected to DNA shuffling techniques such as MOLECULARBREEDING (Maxygen Inc., Santa Clara CA; described in U.S. Patent Number 5,837,458; Chang, C.-C. et al. (1999) Nat. Biotechnol. 17:793-797; Christians, F.C. et al. (1999) Nat. Biotechnol. 17:259-264; and Cramer, A. et al. (1996) Nat. Biotechnol. 14:315-319) to alter or improve the biological properties of EXCS, such as its biological or enzymatic activity or its ability to bind to other molecules or compounds. DNA shuffling is a process by which a library of gene variants is produced using PCR-mediated recombination of gene fragments. The library is then subjected to selection or screening procedures that identify those gene variants with the desired properties. These preferred variants may then be pooled and further subjected to recursive rounds of DNA shuffling and selection/screening. Thus, genetic diversity is created through "artificial" breeding and rapid molecular evolution. For example, fragments of a single gene containing random point mutations may be recombined, screened, and then reshuffled until the desired properties are optimized. Alternatively, fragments of a given gene may be recombined with fragments of homologous genes in the same gene family, either from the same or different species, thereby maximizing the genetic diversity of multiple naturally occurring genes in a directed and controllable manner.

In another embodiment, sequences encoding EXCS may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucleic Acids Symp. Ser. 7:215-223; and Horn, T. et al. (1980) Nucleic Acids Symp. Ser. 7:225-232.) Alternatively, EXCS itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solid-phase techniques. (See, e.g., Roberge, J.Y. et al. (1995) Science 269:202-204.) Automated synthesis may be achieved using the ABI 431A peptide synthesizer (Perkin-Elmer). Additionally, the amino acid sequence of EXCS, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) Methods Enzymol. 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties. WH Freeman, New York NY.)

In order to express a biologically active EXCS, the nucleotide sequences encoding EXCS or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in polynucleotide sequences encoding EXCS. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding EXCS. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding EXCS and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162.)

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding EXCS and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences encoding EXCS. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. The invention is not limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding EXCS. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding EXCS can be achieved using a multifunctional E. coli vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or PSPORT1 plasmid (Life Technologies). Ligation of sequences encoding EXCS into the vector's multiple

cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J. Biol.

- 5 Chem. 264:5503-5509.) When large quantities of EXCS are needed, e.g. for the production of antibodies, vectors which direct high level expression of EXCS may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

- Yeast expression systems may be used for production of EXCS. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH  
10 promoters, may be used in the yeast *Saccharomyces cerevisiae* or *Pichia pastoris*. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 1995, supra; Bitter, G.A. et al. (1987) Methods Enzymol. 153:516-544; and Scorer, C.A. et al. (1994) Bio/Technology 12:181-184.)

- 15 Plant systems may also be used for expression of EXCS. Transcription of sequences encoding EXCS may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al.  
20 (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196.)

- In mammalian cells, a number of viral-based expression systems may be utilized. In cases  
25 where an adenovirus is used as an expression vector, sequences encoding EXCS may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses EXCS in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma  
30 virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

- Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino  
35 polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet.

For long term production of recombinant proteins in mammalian systems, stable expression of EXCS in cell lines is preferred. For example, sequences encoding EXCS can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous  
5 expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using  
10 tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk* and *ap<sup>r</sup>* cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic,  
15 or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* and *par* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. USA 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which  
20 alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP; Clontech),  $\beta$  glucuronidase and its substrate  $\beta$ -glucuronide, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system.  
25 (See, e.g., Rhodes, C.A. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding EXCS is inserted within a marker gene sequence, transformed cells containing sequences encoding EXCS can be identified by the absence of marker gene function. Alternatively, a  
30 marker gene can be placed in tandem with a sequence encoding EXCS under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding EXCS and that express EXCS may be identified by a variety of procedures known to those of skill in the art. These  
35 procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR

amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of EXCS using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on EXCS is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St. Paul MN, Sect. IV; Coligan, J.E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York NY; and Pound, J.D. (1998) Immunochemical Protocols, Humana Press, Totowa NJ.)

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding EXCS include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding EXCS, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Amersham Pharmacia Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding EXCS may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode EXCS may be designed to contain signal sequences which direct secretion of EXCS through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" or "pro" form of the protein may also be used to specify protein targeting, folding, and/or activity.



Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38) are available from the American Type Culture Collection (ATCC, Manassas VA) and may be chosen to ensure the correct modification and processing of the foreign protein.

5 In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding EXCS may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric EXCS protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of EXCS activity. Heterologous protein and  
10 peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and  
15 metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the EXCS encoding sequence and the heterologous protein sequence, so that EXCS may be cleaved away from the heterologous moiety following purification.  
20 Methods for fusion protein expression and purification are discussed in Ausubel (1995, supra, ch. 10). A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled EXCS may be achieved in vitro using the TNT rabbit reticulocyte lysate or wheat germ extract system (Promega). These  
25 systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, for example, <sup>35</sup>S-methionine.

Fragments of EXCS may be produced not only by recombinant means, but also by direct peptide synthesis using solid-phase techniques. (See, e.g., Creighton, supra, pp. 55-60.) Protein  
30 synthesis may be performed by manual techniques or by automation. Automated synthesis may be achieved, for example, using the ABI 431A peptide synthesizer (Perkin-Elmer). Various fragments of EXCS may be synthesized separately and then combined to produce the full length molecule.

#### THERAPEUTICS

Chemical and structural similarity, e.g., in the context of sequences and motifs, exists  
35 between regions of EXCS and extracellular signaling molecules. In addition, the expression of EXCS

is closely associated with reproductive, cardiovascular, nervous, gastrointestinal, cancerous, hematopoietic/immune, cell proliferative and inflamed tissue. Therefore, EXCS appears to play a role in infections and gastrointestinal, neurological, reproductive, autoimmune/inflammatory, and cell proliferative disorders including cancer. In the treatment of disorders associated with increased  
5 EXCS expression or activity, it is desirable to decrease the expression or activity of EXCS. In the treatment of disorders associated with decreased EXCS expression or activity, it is desirable to increase the expression or activity of EXCS.

Therefore, in one embodiment, EXCS or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or  
10 activity of EXCS. Examples of such disorders include, but are not limited to, an infection caused by a parasite classified as plasmodium or malaria-causing, parasitic entamoeba, leishmania, trypanosoma, toxoplasma, pneumocystis carinii, intestinal protozoa such as giardia, trichomonas, tissue nematode such as trichinella, intestinal nematode such as ascaris, lymphatic filarial nematode, trematode such as schistosoma, and cestode such as tapeworm an infection caused by a viral agent  
15 classified as adenovirus, arenavirus, bunyavirus, calicivirus, coronavirus, filovirus, hepadnavirus, herpesvirus, flavivirus, orthomyxovirus, parvovirus, papovavirus, paramyxovirus, picornavirus, poxvirus, reovirus, retrovirus, rhabdovirus, or togavirus; an infection caused by a bacterial agent classified as pneumococcus, staphylococcus, streptococcus, bacillus, corynebacterium, clostridium, meningococcus, gonococcus, listeria, moraxella, kingella, haemophilus, legionella, bordetella, gram-  
20 negative enterobacterium including shigella, salmonella, or campylobacter, pseudomonas, vibrio, brucella, francisella, yersinia, bartonella, norcardium, actinomyces, mycobacterium, spirochaetale, rickettsia, chlamydia, or mycoplasma; an infection caused by a fungal agent classified as aspergillus, blastomyces, dermatophytes, cryptococcus, coccidioides, malassezia, histoplasma, or other mycosis-causing fungal agent; a gastrointestinal disorder such as dysphagia, peptic esophagitis, esophageal  
25 spasm, esophageal stricture, esophageal carcinoma, dyspepsia, indigestion, gastritis, gastric carcinoma, anorexia, nausea, emesis, gastroparesis, antral or pyloric edema, abdominal angina, pyrosis, gastroenteritis, intestinal obstruction, infections of the intestinal tract, peptic ulcer, cholelithiasis, cholecystitis, cholestasis, pancreatitis, pancreatic carcinoma, biliary tract disease, hepatitis, hyperbilirubinemia, cirrhosis, passive congestion of the liver, hepatoma, infectious colitis,  
30 ulcerative colitis, ulcerative proctitis, Crohn's disease, Whipple's disease, Mallory-Weiss syndrome, colonic carcinoma, colonic obstruction, irritable bowel syndrome, short bowel syndrome, diarrhea, constipation, gastrointestinal hemorrhage, acquired immunodeficiency syndrome (AIDS) enteropathy, jaundice, hepatic encephalopathy, hepatorenal syndrome, hepatic steatosis, hemochromatosis, Wilson's disease, alpha<sub>1</sub>-antitrypsin deficiency, Reye's syndrome, primary  
35 sclerosing cholangitis, liver infarction, portal vein obstruction and thrombosis, centrilobular necrosis,

- peliosis hepatis, hepatic vein thrombosis, veno-occlusive disease, preeclampsia, eclampsia, acute fatty liver of pregnancy, intrahepatic cholestasis of pregnancy, and hepatic tumors including nodular hyperplasias, adenomas, and carcinomas; a neurological disorder such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease.
- 5 Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease; prion diseases
- 10 including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome; fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord
- 15 diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system disorders, dermatomyositis and polymyositis; inherited, metabolic, endocrine, and toxic myopathies; myasthenia gravis, periodic paralysis; mental disorders including mood, anxiety, and schizophrenic disorders; seasonal affective disorder (SAD); akathisia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, Tourette's disorder,
- 20 progressive supranuclear palsy, corticobasal degeneration, and familial frontotemporal dementia; a reproductive disorder such as a disorder of prolactin production, infertility, including tubal disease, ovulatory defects, and endometriosis, a disruption of the estrous cycle, a disruption of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, an endometrial or ovarian tumor, a uterine fibroid, autoimmune disorders, an ectopic pregnancy, and teratogenesis; cancer of
- 25 the breast, fibrocystic breast disease, and galactorrhea; a disruption of spermatogenesis, abnormal sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease, impotence, carcinoma of the male breast, and gynecomastia; an autoimmune/inflammatory disorder such as inflammation, actinic keratosis, acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome,
- 30 allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, bursitis, cirrhosis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis.
- 35 Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, paroxysmal nocturnal

- hemoglobinemia, hepatitis, episodic lymphopenia with lymphocytotoxins, mixed connective tissue disease (MCTD), myelofibrosis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, polycythemia vera, primary thrombocythemia, Reiter's syndrome,
- 5 rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma and hematopoietic cancer including lymphoma, leukemia, and myeloma, a cell proliferative disorder such as actinic keratosis,
- 10 arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver,
- 15 lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus.

In another embodiment, a vector capable of expressing EXCS or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of EXCS including, but not limited to, those described above.

- 20 In a further embodiment, a pharmaceutical composition comprising a substantially purified EXCS in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of EXCS including, but not limited to, those provided above.

- In still another embodiment, an agonist which modulates the activity of EXCS may be  
25 administered to a subject to treat or prevent a disorder associated with decreased expression or activity of EXCS including, but not limited to, those listed above.

- In a further embodiment, an antagonist of EXCS may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of EXCS. Examples of such disorders include, but are not limited to, those infections and gastrointestinal, neurological,  
30 reproductive, autoimmune/inflammatory, and cell proliferative disorders including cancer described above. In one aspect, an antibody which specifically binds EXCS may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues which express EXCS.

- In an additional embodiment, a vector expressing the complement of the polynucleotide  
35 encoding EXCS may be administered to a subject to treat or prevent a disorder associated with

increased expression or activity of EXCS including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made  
5 by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of EXCS may be produced using methods which are generally known in the  
10 art. In particular, purified EXCS may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind EXCS. Antibodies to EXCS may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit  
15 dimer formation) are generally preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with EXCS or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral  
20 gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to EXCS have an amino acid sequence consisting of at least about 5 amino acids, and generally will  
25 consist of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of EXCS amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

30 Monoclonal antibodies to EXCS may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. USA 80:2026-2030; and  
35 Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. USA 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda, S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce EXCS-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton, D.R. (1991) Proc. Natl. Acad. Sci. USA 88:10134-10137.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. USA 86:3833-3837; Winter, G. et al. (1991) Nature 349:293-299.)

Antibody fragments which contain specific binding sites for EXCS may also be generated. For example, such fragments include, but are not limited to,  $F(ab')_2$  fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the  $F(ab')_2$  fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) Science 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between EXCS and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering EXCS epitopes is generally used, but a competitive binding assay may also be employed (Pound, supra).

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies for EXCS. Affinity is expressed as an association constant,  $K_a$ , which is defined as the molar concentration of EXCS-antibody complex divided by the molar concentrations of free antigen and free antibody under equilibrium conditions. The  $K_a$  determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple EXCS epitopes, represents the average affinity, or avidity, of the antibodies for EXCS. The  $K_a$  determined for a preparation of monoclonal antibodies, which are monospecific for a particular EXCS epitope, represents a true measure of affinity. High-affinity antibody preparations with  $K_a$  ranging from about  $10^9$  to  $10^{12}$  L/mole are preferred for use in immunoassays in which the

EXCS-antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with  $K_d$  ranging from about  $10^6$  to  $10^7$  L/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of EXCS, preferably in active form, from the antibody (Catty, D. (1988) Antibodies. Volume I: A Practical Approach, IRL Press, Washington, DC;

- 5 Liddell, J.E. and Cryer, A. (1991) A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, New York NY).

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml.

- 10 preferably 5-10 mg specific antibody/ml, is generally employed in procedures requiring precipitation of EXCS-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, supra, and Coligan et al. supra.)

- In another embodiment of the invention, the polynucleotides encoding EXCS, or any  
15 fragment or complement thereof, may be used for therapeutic purposes. In one aspect, the complement of the polynucleotide encoding EXCS may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding EXCS. Thus, complementary molecules or fragments may be used to modulate EXCS activity, or to achieve regulation of gene function. Such  
20 technology is now well known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding EXCS.

- Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted  
25 organ, tissue, or cell population. Methods which are well known to those skilled in the art can be used to construct vectors to express nucleic acid sequences complementary to the polynucleotides encoding EXCS. (See, e.g., Sambrook, supra; Ausubel, 1995, supra.)

- Genes encoding EXCS can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide, or fragment thereof, encoding EXCS. Such  
30 constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector, and may last even longer if appropriate replication elements are part of the vector system.

- 35 As mentioned above, modifications of gene expression can be obtained by designing

complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5' or regulatory regions of the gene encoding EXCS. Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, may be employed. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing, Mt. Kisco NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding EXCS.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding EXCS. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine.



queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nat. Biotechnol. 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as humans, dogs, cats, cows, horses, rabbits, and monkeys.

An additional embodiment of the invention relates to the administration of a pharmaceutical or sterile composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of EXCS, antibodies to EXCS, and mimetics, agonists, antagonists, or inhibitors of EXCS. The compositions may be administered alone or in combination with at least one other agent, such as a stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs, or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing, Easton PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after

grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic

acids. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preparation may be a lyophilized powder which may contain any or all of the following: 1 mM to 50 mM histidine, 0.1% to 2% sucrose, and 2% to 7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

5       After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of EXCS, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The  
10       determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models such as mice, rats, rabbits, dogs, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for  
15       administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example EXCS or fragments thereof, antibodies of EXCS, and agonists, antagonists or inhibitors of EXCS, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by  
20       calculating the  $ED_{50}$  (the dose therapeutically effective in 50% of the population) or  $LD_{50}$  (the dose lethal to 50% of the population) statistics. The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the  $LD_{50}/ED_{50}$  ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such  
25       compositions is preferably within a range of circulating concentrations that includes the  $ED_{50}$  with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the  
30       active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular  
35       formulation.

Normal dosage amounts may vary from about 0.1  $\mu\text{g}$  to 100,000  $\mu\text{g}$ , up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

## DIAGNOSTICS

In another embodiment, antibodies which specifically bind EXCS may be used for the diagnosis of disorders characterized by expression of EXCS, or in assays to monitor patients being treated with EXCS or agonists, antagonists, or inhibitors of EXCS. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for EXCS include methods which utilize the antibody and a label to detect EXCS in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring EXCS, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of EXCS expression. Normal or standard values for EXCS expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, for example, human subjects, with antibody to EXCS under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, such as photometric means. Quantities of EXCS expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding EXCS may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantify gene expression in biopsied tissues in which expression of EXCS may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of EXCS, and to monitor regulation of EXCS levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding EXCS or closely related molecules may be used to identify nucleic acid sequences which encode EXCS. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification will determine whether the probe identifies only naturally occurring sequences encoding EXCS, allelic variants, or related

sequences.

Probes may also be used for the detection of related sequences, and may have at least 50% sequence identity to any of the EXCS encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:27-52 or from  
 5 genomic sequences including promoters, enhancers, and introns of the EXCS gene.

Means for producing specific hybridization probes for DNAs encoding EXCS include the cloning of polynucleotide sequences encoding EXCS or EXCS derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA  
 10 polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as  $^{32}\text{P}$  or  $^{35}\text{S}$ , or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding EXCS may be used for the diagnosis of disorders associated with expression of EXCS. Examples of such disorders include, but are not limited to, an  
 15 infection caused by a parasite classified as plasmodium or malaria-causing, parasitic entamoeba, leishmania, trypanosoma, toxoplasma, pneumocystis carinii, intestinal protozoa such as giardia, trichomonas, tissue nematode such as trichinella, intestinal nematode such as ascaris, lymphatic filarial nematode, trematode such as schistosoma, and cestode such as tapeworm an infection caused by a viral agent classified as adenovirus, arenavirus, bunyavirus, calicivirus, coronavirus, filovirus,  
 20 hepadnavirus, herpesvirus, flavivirus, orthomyxovirus, parvovirus, papovavirus, paramyxovirus, picornavirus, poxvirus, reovirus, retrovirus, rhabdovirus, or togavirus; an infection caused by a bacterial agent classified as pneumococcus, staphylococcus, streptococcus, bacillus, corynebacterium, clostridium, meningococcus, gonococcus, listeria, moraxella, kingella, haemophilus, legionella, bordetella, gram-negative enterobacterium including shigella, salmonella, or campylobacter,  
 25 pseudomonas, vibrio, brucella, francisella, yersinia, bartonella, norcardium, actinomyces, mycobacterium, spirochaetale, rickettsia, chlamydia, or mycoplasma; an infection caused by a fungal agent classified as aspergillus, blastomyces, dermatophytes, cryptococcus, coccidioides, malassezia, histoplasma, or other mycosis-causing fungal agent; a gastrointestinal disorder such as dysphagia, peptic esophagitis, esophageal spasm, esophageal stricture, esophageal carcinoma, dyspepsia,  
 30 indigestion, gastritis, gastric carcinoma, anorexia, nausea, emesis, gastroparesis, antral or pyloric edema, abdominal angina, pyrosis, gastroenteritis, intestinal obstruction, infections of the intestinal tract, peptic ulcer, cholelithiasis, cholecystitis, cholestasis, pancreatitis, pancreatic carcinoma, biliary tract disease, hepatitis, hyperbilirubinemia, cirrhosis, passive congestion of the liver, hepatoma, infectious colitis, ulcerative colitis, ulcerative proctitis, Crohn's disease, Whipple's disease, Mallory-  
 35 Weiss syndrome, colonic carcinoma, colonic obstruction, irritable bowel syndrome, short bowel

syndrome, diarrhea, constipation, gastrointestinal hemorrhage, acquired immunodeficiency syndrome (AIDS) enteropathy, jaundice, hepatic encephalopathy, hepatorenal syndrome, hepatic steatosis, hemochromatosis, Wilson's disease, alpha<sub>1</sub>-antitrypsin deficiency, Reye's syndrome, primary sclerosing cholangitis, liver infarction, portal vein obstruction and thrombosis, centrilobular necrosis,

5 peliosis hepatis, hepatic vein thrombosis, veno-occlusive disease, preeclampsia, eclampsia, acute fatty liver of pregnancy, intrahepatic cholestasis of pregnancy, and hepatic tumors including nodular hyperplasias, adenomas, and carcinomas; a neurological disorder such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic

10 lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease; prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome; fatal

15 familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system

20 disorders, dermatomyositis and polymyositis; inherited, metabolic, endocrine, and toxic myopathies; myasthenia gravis, periodic paralysis; mental disorders including mood, anxiety, and schizophrenic disorders; seasonal affective disorder (SAD); akathisia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, Tourette's disorder, progressive supranuclear palsy, corticobasal degeneration, and familial frontotemporal dementia; a

25 reproductive disorder such as a disorder of prolactin production, infertility, including tubal disease, ovulatory defects, and endometriosis, a disruption of the estrous cycle, a disruption of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, an endometrial or ovarian tumor, a uterine fibroid, autoimmune disorders, an ectopic pregnancy, and teratogenesis; cancer of the breast, fibrocystic breast disease, and galactorrhea; a disruption of spermatogenesis, abnormal

30 sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease, impotence, carcinoma of the male breast, and gynecomastia; an autoimmune/inflammatory disorder such as inflammation, actinic keratosis, acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune

35 hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal

dystrophy (APECED). bronchitis. bursitis. cirrhosis. cholecystitis. contact dermatitis. Crohn's disease. atopic dermatitis. dermatomyositis. diabetes mellitus. emphysema. episodic lymphopenia with lymphocytotoxins. erythroblastosis fetalis. erythema nodosum. atrophic gastritis. glomerulonephritis. Goodpasture's syndrome. gout. Graves' disease. Hashimoto's thyroiditis. paroxysmal nocturnal hemoglobinemia. hepatitis. episodic lymphopenia with lymphocytotoxins. mixed connective tissue disease (MCTD). myelofibrosis. hypereosinophilia. irritable bowel syndrome. multiple sclerosis. myasthenia gravis. myocardial or pericardial inflammation. osteoarthritis. osteoporosis. pancreatitis. polymyositis. psoriasis. polycythemia vera. primary thrombocythemia. Reiter's syndrome. rheumatoid arthritis. scleroderma. Sjögren's syndrome. systemic anaphylaxis. systemic lupus erythematosus. systemic sclerosis. thrombocytopenic purpura. ulcerative colitis. uveitis. Werner syndrome, complications of cancer. hemodialysis. and extracorporeal circulation. viral. bacterial. fungal. parasitic. protozoal. and helminthic infections, and trauma and hematopoietic cancer including lymphoma. leukemia. and myeloma, a cell proliferative disorder such as actinic keratosis, arteriosclerosis. atherosclerosis. bursitis. cirrhosis. hepatitis. mixed connective tissue disease (MCTD). myelofibrosis. paroxysmal nocturnal hemoglobinuria. polycythemia vera. psoriasis. primary thrombocythemia, and cancers including adenocarcinoma. leukemia. lymphoma. melanoma. myeloma. sarcoma. teratocarcinoma. and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands. skin, spleen, testis, thymus, thyroid, and uterus. The polynucleotide sequences encoding EXCS may be used in Southern or northern analysis. dot blot. or other membrane-based technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like assays; and in microarrays utilizing fluids or tissues from patients to detect altered EXCS expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding EXCS may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding EXCS may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantified and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding EXCS in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of

EXCS, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding EXCS, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of an abnormal amount of transcript (either under- or overexpressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding EXCS may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding EXCS, or a fragment of a polynucleotide complementary to the polynucleotide encoding EXCS, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantification of closely related DNA or RNA sequences.

Methods which may also be used to quantify the expression of EXCS include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) J. Immunol. Methods 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 212:229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in a high-throughput format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as targets in a microarray. The microarray



can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents.

5           Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci. USA 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. USA 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.)

10           In another embodiment of the invention, nucleic acid sequences encoding EXCS may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single  
15           chromosome cDNA libraries. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355; Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.)

          Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, supra, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the  
20           Online Mendelian Inheritance in Man (OMIM) World Wide Web site. Correlation between the location of the gene encoding EXCS on a physical chromosomal map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The nucleotide sequences of the invention may be used to detect differences in gene sequences among normal, carrier, and affected individuals.

25           In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms by physical mapping. This provides  
30           valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequence of the subject invention may also  
35           be used to detect differences in the chromosomal location due to translocation, inversion, etc., among

normal, carrier, or affected individuals.

In another embodiment of the invention, EXCS, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between EXCS and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate. The test compounds are reacted with EXCS, or fragments thereof, and washed. Bound EXCS is then detected by methods well known in the art. Purified EXCS can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding EXCS specifically compete with a test compound for binding EXCS. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with EXCS.

In additional embodiments, the nucleotide sequences which encode EXCS may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The disclosures of all patents, applications and publications, mentioned above and below, in particular U.S. Ser. No. 60/134,949, U.S. Ser. No. 60/144,270, U.S. Ser. No. 60/146,700, and U.S. Ser. No. 60/157,508, are hereby expressly incorporated by reference.

## EXAMPLES

### I. Construction of cDNA Libraries

RNA was purchased from Clontech or isolated from tissues described in Table 4. Some tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged

over CsCl cushions or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods.

Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. In some cases, RNA was treated with DNase. For most libraries, poly(A+) RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX latex particles (QIAGEN, Chatsworth CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g., the POLY(A)PURE mRNA purification kit (Ambion, Austin TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP vector system (Stratagene) or SUPERScript plasmid system (Life Technologies), using the recommended procedures or similar methods known in the art. (See, e.g., Ausubel, 1997, supra, units 5.1-6.6.) Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column chromatography (Amersham Pharmacia Biotech) or preparative agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g., PBLUESCRIPT plasmid (Stratagene), PSPO1 plasmid (Life Technologies), pcDNA2.1 plasmid (Invitrogen, Carlsbad CA), or pINCY plasmid (Incyte Pharmaceuticals, Palo Alto CA). Recombinant plasmids were transformed into competent *E. coli* cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5 $\alpha$ , DH10B, or ElectroMAX DH10B from Life Technologies.

## II. Isolation of cDNA Clones

Plasmids were recovered from host cells by in vivo excision using the UNIZAP vector system (Stratagene) or by cell lysis. Plasmids were purified using at least one of the following: a Magic or WIZARD Minipreps DNA purification system (Promega); an AGTC Miniprep purification kit (Edge Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, QIAWELL 8 Plus Plasmid, QIAWELL 8 Ultra Plasmid purification systems or the R.E.A.L. PREP 96 plasmid purification kit from QIAGEN. Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a high-throughput format (Rao, V.B. (1994) Anal. Biochem. 216:1-14). Host cell lysis and thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN dye (Molecular Probes, Eugene OR) and a FLUOROSKAN II fluorescence

scanner (LabSystems Oy, Helsinki, Finland).

### III. Sequencing and Analysis

cDNA sequencing reactions were processed using standard methods or high-throughput instrumentation such as the ABI CATALYST 800 (Perkin-Elmer) thermal cycler or the PTC-200  
5 thermal cycler (MJ Research) in conjunction with the HYDRA microdispenser (Robbins Scientific) or the MICROLAB 2200 (Hamilton) liquid transfer system. cDNA sequencing reactions were prepared using reagents provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer). Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides  
10 were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the ABI PRISM 373 or 377 sequencing system (Perkin-Elmer) in conjunction with standard ABI protocols and base calling software; or other sequence analysis systems known in the art. Reading frames within the cDNA sequences were identified using standard methods (reviewed in Ausubel, 1997, *supra*, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques  
15 disclosed in Example V.

The polynucleotide sequences derived from cDNA sequencing were assembled and analyzed using a combination of software programs which utilize algorithms well known to those skilled in the art. Table 5 summarizes the tools, programs, and algorithms used and provides applicable descriptions, references, and threshold parameters. The first column of Table 5 shows the tools,  
20 programs, and algorithms used, the second column provides brief descriptions thereof, the third column presents appropriate references, all of which are incorporated by reference herein in their entirety, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match between two sequences (the higher the score, the greater the homology between two sequences). Sequences were analyzed using MACDNASIS PRO  
25 software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Polynucleotide and polypeptide sequence alignments were generated using the default parameters specified by the clustal algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.

30 The polynucleotide sequences were validated by removing vector, linker, and polyA sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programming, and dinucleotide nearest neighbor analysis. The sequences were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS, PRINTS, DOMO, PRODOM, and PFAM to acquire  
35 annotation using programs based on BLAST, FASTA, and BLIMPS. The sequences were assembled

into full length polynucleotide sequences using programs based on Phred, Phrap, and Consed, and were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length amino acid sequences, and these full length sequences were subsequently analyzed by querying  
5 against databases such as the GenBank databases (described above), SwissProt, BLOCKS, PRINTS, DOMO, PRODOM, Prosite, and Hidden Markov Model (HMM)-based protein family databases such as PFAM. HMM is a probabilistic approach which analyzes consensus primary structures of gene families. (See, e.g., Eddy, S.R. (1996) Curr. Opin. Struct. Biol. 6:361-365.)

The programs described above for the assembly and analysis of full length polynucleotide  
10 and amino acid sequences were also used to identify polynucleotide sequence fragments from SEQ ID NO:27-52. Fragments from about 20 to about 4000 nucleotides which are useful in hybridization and amplification technologies were described in The Invention section above.

#### IV. Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a  
15 gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, supra, ch. 7; Ausubel, 1995, supra, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in nucleotide databases such as GenBank or LIFESEQ (Incyte Pharmaceuticals). This  
20 analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score, which is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

25 The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1% to 2% error, and, with a product score of 70, the match will be exact. Similar molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

30 The results of northern analyses are reported as a percentage distribution of libraries in which the transcript encoding EXCS occurred. Analysis involved the categorization of cDNA libraries by organ/tissue and disease. The organ/tissue categories included cardiovascular, dermatologic, developmental, endocrine, gastrointestinal, hematopoietic/immune, musculoskeletal, nervous, reproductive, and urologic. The disease/condition categories included cancer, inflammation, trauma,  
35 cell proliferation, neurological, and pooled. For each category, the number of libraries expressing the

sequence of interest was counted and divided by the total number of libraries across all categories. Percentage values of tissue-specific and disease- or condition-specific expression are reported in Table 3.

#### V. Chromosomal Mapping of EXCS Encoding Polynucleotides

5 The cDNA sequences which were used to assemble SEQ ID NO:45-52 were compared with sequences from the Incyte LIFESEQ database and public domain databases using BLAST and other implementations of the Smith-Waterman algorithm. Sequences from these databases that matched SEQ ID NO:27-52 were assembled into clusters of contiguous and overlapping sequences using assembly algorithms such as Phrap (Table 5). Radiation hybrid and genetic mapping data available  
10 from public resources such as the Stanford Human Genome Center (SHGC), Whitehead Institute for Genome Research (WIGR), and Généthon were used to determine if any of the clustered sequences had been previously mapped. Inclusion of a mapped sequence in a cluster resulted in the assignment of all sequences of that cluster, including its particular SEQ ID NO:, to that map location.

The genetic map location of SEQ ID NO:47 is described in The Invention as a range, or  
15 interval, of a human chromosome. The map position of an interval, in centiMorgans, is measured relative to the terminus of the chromosome's p-arm. (The centiMorgan (cM) is a unit of measurement based on recombination frequencies between chromosomal markers. On average, 1 cM is roughly equivalent to 1 megabase (Mb) of DNA in humans, although this can vary widely due to hot and cold spots of recombination.) The cM distances are based on genetic markers mapped by Généthon which  
20 provide boundaries for radiation hybrid markers whose sequences were included in each of the clusters. Human genome maps and other resources available to the public, such as the NCBI "GeneMap'99" World Wide Web site (<http://www.ncbi.nlm.nih.gov/genemap/>), can be employed to determine if previously identified disease genes map within or in proximity to the intervals indicated above.

#### 25 VI. Extension of EXCS Encoding Polynucleotides

The full length nucleic acid sequences of SEQ ID NO:27-52 were produced by extension of an appropriate fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other primer, to initiate 3' extension of the known fragment. The initial primers were designed using  
30 OLIGO 4.06 software (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries were used to extend the sequence. If more than one  
35 extension was necessary or desired, additional or nested sets of primers were designed.

High fidelity amplification was obtained by PCR using methods well known in the art. PCR was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction mix contained DNA template, 200 nmol of each primer, reaction buffer containing  $Mg^{2+}$ ,  $(NH_4)_2SO_4$ , and  $\beta$ -mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and-PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the alternative, the parameters for primer pair T7 and SK+ were as follows: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well was determined by dispensing 100  $\mu$ l PICOGREEN quantitation reagent (0.25% (v/v) PICOGREEN; Molecular Probes, Eugene OR) dissolved in 1X TE and 0.5  $\mu$ l of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar, Acton MA), allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II (Labsystems Oy, Helsinki, Finland) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5  $\mu$ l to 10  $\mu$ l aliquot of the reaction mixture was analyzed by electrophoresis on a 1 % agarose mini-gel to determine which reactions were successful in extending the sequence.

The extended nucleotides were desalted and concentrated, transferred to 384-well plates, digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For shotgun sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones were religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into competent E. coli cells. Transformed cells were selected on antibiotic-containing media, individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA polymerase (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted with 20% dimethylsulfoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing

primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer).

In like manner, the nucleotide sequences of SEQ ID NO:27-52 are used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for such extension, and an appropriate genomic library.

## VII. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:27-52 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250  $\mu$ Ci of [ $\gamma$ - $^{32}$ P] adenosine triphosphate (Amersham Pharmacia Biotech), and T4 polynucleotide kinase (DuPont NEN, Boston MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (Amersham Pharmacia Biotech). An aliquot containing  $10^7$  counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under conditions of up to, for example, 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. Hybridization patterns are visualized using autoradiography or an alternative imaging means and compared.

## VIII. Microarrays

A chemical coupling procedure and an ink jet device can be used to synthesize array elements on the surface of a substrate. (See, e.g., Baldeschweiler, *supra*.) An array analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced by hand or using available methods and machines and contain any appropriate number of elements. After hybridization, nonhybridized probes are removed and a scanner used to determine the levels and patterns of fluorescence. The degree of complementarity and the relative abundance of each probe which hybridizes to an element on the microarray may be assessed through analysis of the scanned images.

Full-length cDNAs, Expressed Sequence Tags (ESTs), or fragments thereof may comprise the elements of the microarray. Fragments suitable for hybridization can be selected using software



well known in the art such as LASERGENE software (DNASTAR). Full-length cDNAs, ESTs, or fragments thereof corresponding to one of the nucleotide sequences of the present invention, or selected at random from a cDNA library relevant to the present invention, are arranged on an appropriate substrate, e.g., a glass slide. The cDNA is fixed to the slide using, e.g., UV cross-linking followed by thermal and chemical treatments and subsequent drying. (See, e.g., Schena, M. et al. (1995) Science 270:467-470; Shalon, D. et al. (1996) Genome Res. 6:639-645.) Fluorescent probes are prepared and used for hybridization to the elements on the substrate. The substrate is analyzed by procedures described above.

#### IX. Complementary Polynucleotides

Sequences complementary to the EXCS-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring EXCS. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO 4.06 software (National Biosciences) and the coding sequence of EXCS. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the EXCS-encoding transcript.

#### X. Expression of EXCS

Expression and purification of EXCS is achieved using bacterial or virus-based expression systems. For expression of EXCS in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac (tac)* hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express EXCS upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of EXCS in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding EXCS by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E.K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther.

In most expression systems, EXCS is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Amersham Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from EXCS at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995, supra, ch. 10 and 16). Purified EXCS obtained by these methods can be used directly in the following activity assay.

#### **XI. Demonstration of EXCS Activity**

EXCS activity is measured by one of several methods. Growth factor activity is measured by the stimulation of DNA synthesis in Swiss mouse 3T3 cells. (McKay, I. and Leigh, I., eds. (1993) Growth Factors: A Practical Approach, Oxford University Press, New York, NY.) Initiation of DNA synthesis indicates the cells' entry into the mitotic cycle and their commitment to undergo later division. 3T3 cells are competent to respond to most growth factors, not only those that are mitogenic, but also those that are involved in embryonic induction. This competence is possible because the in vivo specificity demonstrated by some growth factors is not necessarily inherent but is determined by the responding tissue. In this assay, varying amounts of EXCS are added to quiescent 3T3 cultured cells in the presence of [<sup>3</sup>H]thymidine, a radioactive DNA precursor. EXCS for this assay can be obtained by recombinant means or from biochemical preparations. Incorporation of [<sup>3</sup>H]thymidine into acid-precipitable DNA is measured over an appropriate time interval, and the amount incorporated is directly proportional to the amount of newly synthesized DNA. A linear dose-response curve over at least a hundred-fold EXCS concentration range is indicative of growth factor activity. One unit of activity per milliliter is defined as the concentration of EXCS producing a 50% response level, where 100% represents maximal incorporation of [<sup>3</sup>H]thymidine into acid-precipitable DNA.

Alternatively, an assay for cytokine activity measures the proliferation of cultured cells such as fibroblasts or leukocytes. In this assay, the amount of tritiated thymidine incorporated into newly synthesized DNA is used to estimate proliferative activity. Varying amounts of EXCS are added to cultured fibroblasts, or cultured leukocytes such as granulocytes, monocytes, or lymphocytes, in the presence of [<sup>3</sup>H]thymidine, a radioactive DNA precursor. EXCS for this assay can be obtained by

recombinant means or from biochemical preparations. Incorporation of [ $^3\text{H}$ ]thymidine into acid-precipitable DNA is measured over an appropriate time interval, and the amount incorporated is directly proportional to the amount of newly synthesized DNA. A linear dose-response curve over at least a hundred-fold EXCS concentration range is indicative of EXCS activity. One unit of activity per milliliter is conventionally defined as the concentration of EXCS producing a 50% response level, where 100% represents maximal incorporation of [ $^3\text{H}$ ]thymidine into acid-precipitable DNA.

An alternative assay for EXCS cytokine activity utilizes a Boyden micro chamber (Neuroprobe, Cabin John, MD) to measure leukocyte chemotaxis. In this assay, about  $10^5$  migratory cells such as macrophages or monocytes are placed in cell culture media in the upper compartment of the chamber. Varying dilutions of EXCS are placed in the lower compartment. The two compartments are separated by a 5 or 8 micron pore polycarbonate filter (Nucleopore, Pleasanton CA). After incubation at  $37^\circ\text{C}$  for 80 to 120 minutes, the filters are fixed in methanol and stained with appropriate labeling agents. Cells which migrate to the other side of the filter are counted using standard microscopy. The chemotactic index is calculated by dividing the number of migratory cells counted when EXCS is present in the lower compartment by the number of migratory cells counted when only media is present in the lower compartment. The chemotactic index is proportional to the activity of EXCS.

Alternatively, cell lines or tissues transformed with a vector containing nucleotide sequences encoding EXCS can be assayed for EXCS activity by immunoblotting. Cells are denatured in SDS in the presence of  $\beta$ -mercaptoethanol, nucleic acids removed by ethanol precipitation, and proteins purified by acetone precipitation. Pellets are resuspended in 20 mM tris buffer at pH 7.5 and incubated with Protein G-Sepharose pre-coated with an antibody specific for EXCS. After washing, the Sepharose beads are boiled in electrophoresis sample buffer, and the eluted proteins subjected to SDS-PAGE. The SDS-PAGE is transferred to a nitrocellulose membrane for immunoblotting, and the EXCS activity is assessed by visualizing and quantifying bands on the blot using the antibody specific for EXCS as the primary antibody and  $^{125}\text{I}$ -labeled IgG specific for the primary antibody as the secondary antibody.

## **XII. Functional Assays**

EXCS function is assessed by expressing the sequences encoding EXCS at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT plasmid (Life Technologies) and pCR3.1 plasmid (Invitrogen), both of which contain the cytomegalovirus promoter. 5-10  $\mu\text{g}$  of recombinant vector are transiently transfected into a human cell line, for example, an endothelial or hematopoietic cell line, using either liposome

formulations or electroporation. 1-2  $\mu$ g of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP;

- 5 Clontech). CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP and to evaluate the apoptotic state of the cells and other cellular properties. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide;
- 10 changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are
- 15 discussed in Ormerod, M.G. (1994) Flow Cytometry, Oxford, New York NY.

The influence of EXCS on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding EXCS and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected

20 cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding EXCS and other genes of interest can be analyzed by northern analysis or microarray techniques.

### **XIII. Production of EXCS Specific Antibodies**

- 25 EXCS substantially purified using polyacrylamide gel electrophoresis (PAGE; see, e.g., Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

- Alternatively, the EXCS amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is
- 30 synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, supra, ch. 11.)

- Typically, oligopeptides of about 15 residues in length are synthesized using an ABI 431A peptide synthesizer (Perkin-Elmer) using fmoc-chemistry and coupled to KLH (Sigma-Aldrich, St.
- 35 Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase

immunogenicity. (See, e.g., Ausubel, 1995. supra.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide and anti-EXCS activity by, for example, binding the peptide or EXCS to a substrate, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

5    **XIV.    Purification of Naturally Occurring EXCS Using Specific Antibodies**

Naturally occurring or recombinant EXCS is substantially purified by immunoaffinity chromatography using antibodies specific for EXCS. An immunoaffinity column is constructed by covalently coupling anti-EXCS antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is  
10   blocked and washed according to the manufacturer's instructions.

Media containing EXCS are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of EXCS (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/EXCS binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such  
15   as urea or thiocyanate ion), and EXCS is collected.

**XV.    Identification of Molecules Which Interact with EXCS**

EXCS, or biologically active fragments thereof, are labeled with <sup>125</sup>I Bolton-Hunter reagent. (See, e.g., Bolton A.E. and W.M. Hunter (1973) Biochem. J. 133:529-539.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled EXCS, washed,  
20   and any wells with labeled EXCS complex are assayed. Data obtained using different concentrations of EXCS are used to calculate values for the number, affinity, and association of EXCS with the candidate molecules.

Alternatively, molecules interacting with EXCS are analyzed using the yeast two-hybrid system as described in Fields, S. and O. Song (1989. Nature 340:245-246), or using commercially  
25   available kits based on the two-hybrid system, such as the MATCHMAKER system (Clontech).

Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with certain embodiments, it  
30   should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

Table 1

Polypeptide SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
1	27	1288847	BRAINOT11	00334U1 (U937NOT01), 840916T6 (PROSTUT05), 1288847F6 (BRAINOT11), 1288847H1 (BRAINOT11), 1651772F6 (PROSTUT08), 2720131F6 (LUNGUT10), 2954659F6 (KIDNFET01), 3321171H1 (PTHYNOT03), 3520878T6 (LUNGNOT03), 3870826H1 (BMARNOT03), 5271406H1 (OVARINOT02), SBYA00334U1
2	28	1329044	PANCNOT07	04082U1 (HMCINOT01), 1329044F1 (PANCNOT07), 1329044H1 (PANCNOT07), 1329044T1 (PANCNOT07), SBYA04082U1
3	29	1493630	PROSNON01	1493630H1 (PROSNON01), 1493630R6 (PROSNON01), 1493630T1 (PROSNON01)
4	30	1533041	SPLNNOT04	1533041F1 (SPLNNOT04), 1533041H1 (SPLNNOT04), 2688779F6 (LUNGNOT23), 3973608H1 (ADRETUT06)
5	31	1566162	HEALDIT02	1566162H1 (HEALDIT02), 1759922T6 (PITUNOT03)
6	32	1811831	PROSTUT12	1811831F6 (PROSTUT12), 1811831H1 (PROSTUT12)
7	33	1835447	BRAINON01	1835447H1 (BRAINON01), 1835447R6 (BRAINON01), 4523747H1 (HNT2TXT01), 5310808H1 (KIDETXS02)
8	34	3892281	BRSTTUT16	1948957R6 (PITUNOT01), 3892281H1 (BRSTTUT16), 3895852T6 (TLYMNOT05)
9	35	4318494	BRADDIT02	4318494F6 (BRADDIT02), 4318494H1 (BRADDIT02), 4318494T6 (BRADDIT02)
10	36	5090841	UTRSTMR01	742729H1 (PANCNOT04), 1329245H1 (PANCNOT07), 4539309H1 (THYRTMT01), 5090841F6 (UTRSTMR01), 5090841H1 (UTRSTMR01), 5153892H1 (HEARFET03)
11	37	2006548	TESTNOT03	1725329X11C1 (PROSNOT14), 2006548H1 (TESTNOT03), 3476792F6 (OVARNOT11), SBIA08125D1, SBIA01870D1
12	38	2207183	SINTFET03	191932F1 (SYNORAB01), 1273270F1 (TESTTUT02), 2207183H1 (SINTFET03), 2219907H1 (LUNGNOT18), 3336344H1 (SPLNNOT10)
13	39	2267403	UTRSNOT02	1449035F1 and 1449035R1 (PLACNOT02), 1599756F6 (BLADNOT03), 2267403H1 and 2267403R6 (UTRSNOT02), 3145756F6 (TESTNOT07)
14	40	2933038	THYMNON04	157761F1 (THP1PLB02), 2933038H1 (THYMNON04), 3294396F6 (TLYJINT01)

Table 1 (cont.)

Polypeptide SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
15	41	3216587	TESTNOT07	3216587F6 and 3216587H2 (TESTNOT07), 3416261H1 (PTHYNOT04), 4204275F6 (BRAITUT29), 4316562F6 (BRAFNOT01), 5385916H1 (BRAINOT19)
16	42	5037143	LIVRTUT13	5035406H1, 5037143H1, and 5037690T6 (LIVRTUT13)
17	43	1235265	LUNGFET03	523352F1 (MMLR2DT01), 1262491R1 (SYNORAT05), 1699607F6 (BLADTUT05), 1717617H1 (UCMCNOT02), 1720254F6 (BLADNOT06), SBLA02298F1
18	44	5571181	TYMNOT08	4348184T6 (TYMNTXT01), 4905349F6 (TYMNOT08), 5571181H1 (TYMNOT08)
19	45	685374	UTRSNOT02	111201R6 (PITUNOT01), 685374H1 (UTRSNOT02), 685374R6 (UTRSNOT02), 837768R1 (PROSNOT07), 1369176R6 (SCORNON02), 3321269H1 (PTHYNOT03), 4309489H1 (BRAUNOT01), 4943366F6 (BRAIFEN05), 5108512H1 (PROSTUS19)
20	46	843193	PROSTUT05	843193H1 (PROSTUT05), 843193X23 (PROSTUT05), 843193X25 (PROSTUT05), 996669R6 (KIDNTUT01), 4699738F6 (BRALNOT01), SZAL00006D1
21	47	1359783	LUNGNOT12	1359783F1 (LUNGNOT12), 1403716H1 (LATRTUT02), 2160063H1 (ENDCNOT02), 2464542H1 (THYRNOT08), 3423249H1 (UCMCNOT04), SANA00380F1, SANA02266F1, SANA02708F1, SANA00364F1, SANA01088F1, SANA03698F1
22	48	1440015	THYRNOT03	1440015H1 (THYRNOT03), 1462822H1 (PANCNOT04), 1577577F6 (LNODNOT03)
23	49	1652885	PROSTUT08	1478195T1 (CORPNOT02), 1652885F6 (PROSTUT08), 1652885H1 (PROSTUT08)
24	50	4003984	HNT2AZS07	4003984H1 (HNT2AZS07), 4003984R6 (HNT2AZS07), 4003984T6 (HNT2AZS07)
25	51	4365383	SKIRNOT01	4365383F6 (SKIRNOT01), 4365383H1 (SKIRNOT01), 5098601H2 (EPIMNON05), 93228929
26	52	5497814	BRABDIR01	5497814F6 (BRABDIR01), 5497814H1 (BRABDIR01)

Table 2

Polypeptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequence	Homologous Sequences	Analytical Methods and Databases
1	77	S62		Signal peptide: M1-T20		SPSCAN HMM
2	88	T3 S23 T59 S65		Signal peptide: M1-E26 or M1-S27		SPSCAN HMM
3	96	S20 S83 S91		Signal peptide: M1-G21 or M1-C22		SPSCAN HMM
4	104	S45 S90		Signal peptide: M1-S30 or M1-C26		SPSCAN HMM
5	60	S19		Signal peptide: M1-S19		SPSCAN HMM
6	117	T105 S40 S112 S40		Signal peptide: M1-A28 or M1-A31		SPSCAN HMM
7	86	S26		Signal peptide: M1-A24 or M1-P22 ATP/GTP binding site: G45-T52		SPSCAN HMM MOTIFS
8	109	S27 S69 S51		Signal peptide: M1-G23 or M1-A29		SPSCAN HMM
9	111	S45 S52 S74		Signal peptide: M1-S20		SPSCAN HMM
10	182	T161 S125 T148		Signal peptide: M1-A34 or M1-S31		SPSCAN HMM
11	105	T15 S64 Y94		Signal Peptide: M1-I22 Venom Protein A: A20-C96	Venom protein A (P25687) g6524951 Bv8 variant 3 precursor	BLAST-GenBank BLAST-SwissProt MOTIFS SPScan BLAST-PRODOM HMMER



Table 2 (cont.)

Polypeptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequence	Homologous Sequences	Analytical Methods and Databases
12	342	S48 S50 T61 T167 S194 S255 S14 S39 S74 T225 T334	N157 N192 N270 N281	Transmembrane Domain: L314-T334 EGF-like domain cysteine pattern signature: C294- C305 EGF domain: D258- Q308	g4689122 HSPC013 hematopoietic stem/progenitor cells	BLAST-GenBank MOTIFS HMMER BLAST - DOMO
13	451	S41 T62 S125 S142 T154 S182 S260 T281 S398 S444 T239 S328 T416	N114	Signal Peptide: M1-A25 ATP/GTP binding site motif A (P- loop): A251-T258 von Willebrand factor type C domains: C33-C95, C111-C174, C252- C314	g4808227 C-terminal part of a Chordin-like protein	BLAST-GenBank MOTIFS SPScan HMMER-PFAM, HMMER BLIMPS - PFAM
14	189	T64 S37		Signal Peptide : M1-P23 Interleukin-6/G- CSF: T65-F109 S151-A181	Y29783 Human interleukin B30	MOTIFS SPScan HMMER BLIMPS-BLOCKS BLIMPS-PRINTS BLAST-GENESEQ
15	216	S19 T49 T122 T191 S198 T49 T73 S105 T170	N47	Recoverin Family Signature: H34- F48, F48-G67, L94-L115, L118- N137, G140-M158, P164-F179, V190- L210 EF-hand Domains: K126-I154, H174- D202	Calsenilin (g4416432) A-type potassium channel modulatory protein 1 (g6969255)	BLAST-GenBank MOTIFS HMMER-PFAM BLIMPS-PRINTS

Table 2 (cont.)

Polypeptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequence	Homologous Sequences	Analytical Methods and Databases
16	178	T111 S174 T124		Signal Peptide: M1-G29 Pancreatic hormone peptide: A30-C65 Pancreatic hormone precursor: G149- L178	Pancreatic polypeptide precursor (g190270)	BLAST-GenBank MOTIFS HMMER SPSCAN ProfileScan HMMER-PFAM BLAST-PRODOM BLIMPS-PRINTS BLIMPS-BLOCKS
17	177	S168 T22 S43 S73 S115 S175	N5	Signal peptide: M1-A60	Fibrosin (g710336) <u>Mus</u> musculus	MOTIFS BLAST-GenBank SPSCAN
18	179	S64 S84 T99 T53 S86 S108 S126 T151 S173	N54 N68 N97	Signal peptide: M1-A33 Transmembrane domain: V8-L27	g6996554 TIF alpha protein	BLAST-GENBANK MOTIFS SPSCAN HMMR
19	213	S7 T39 S93 S155 S187 S112 Y46	N189 N202	G178-S185: ATP/GTP binding site Fibroblast growth factors: K14-P145 HBGF/FGF family signature: V58- S112, W116-P143	g4323515 Fibroblast growth factor 13 isoform 1B	Motifs BLAST-GENBANK HMMER-PFAM PROFILESCAN BLIMPS-BLOCKS BLIMPS-PRINTS BLAST-PRODOM BLAST_DOMO
20	239	S97 T99 T45 S71 S85 T92 S127 S144 T226 T232 Y81 Y130	N160	HBGF/FGF family signature: Q74- L201 Signal peptide: M1-P15 IL1/HBGF Family Signature: D149- H169	g3041790 Fibroblast growth factor FGF-17	Motifs BLAST-GENBANK SPSCAN HMMER-PFAM PROFILESCAN BLIMPS-BLOCKS BLIMPS-PRINTS BLAST-PRODOM BLAST_DOMO

Table 2 (cont.)

Polypeptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequence	Homologous Sequences	Analytical Methods and Databases
21	493	T251 T331 T340 S430 T132 S154 S287 S399 S402 T425 T475	N249	Signal peptide: M1-S17 EGF-like domain: C224-R299	g2429083 T16 EGF-like protein	Motifs BLAST_GENBANK SPSCAN HMMER BLIMPS_BLOCKS BLAST_DOMO
22	121	S76 S76		Signal peptide: M1-D30 Bombesin- like peptides family signature: R46-M56, D30-R81 Bombesin family Neuromedin B Precursor threshold: G57- K121	g 189228 Neuromedin B [Homo sapiens]	Motifs BLAST_GENBANK HMMER SPSCAN BLIMPS_BLOCKS PROFILES SCAN BLAST_PRODOR BLAST_DOMO
23	116	S16 S29 S86 S93			g2232301 FMRamide-related prepropeptide [Homo sapiens]	Motifs BLAST_GENBANK
24	136	T60 S85 S129 S77 Y67	N58	Signal peptide: M1-A19	g6715115 agkisacutacin	BLAST_GENBANK Motifs SPSCAN
25	176	S19 S19 S72 S170 T6 S7 S107 T148		Interleukin-1: I20-Pl63 Interleukin-1 signature: Q92- E158	g6694392 FIL1 (IL1 family protein) zeta	BLAST_GENBANK Motifs HMMER_Pfam PROFILES SCAN BLAST_DOMO
26	134	S102		Signal peptide: M1-H18		Motifs HMMER

Table 3

Nucleotide SEQ ID NO:	Selected Fragments	Tissue Expression (Fraction of Total)	Disease or Condition (Fraction of Total)	Vector
27	651-695	Nervous (0.321) Cardiovascular (0.143) Developmental (0.143)	Cancer (0.357) Inflammation (0.250) Fetal/Cell Proliferation (0.214)	pINCY
28	271-315	Developmental (1.000)	Fetal/Cell Proliferation (1.000)	pINCY
29	327-371	Reproductive (1.000)	Cancer (0.667) Trauma (0.333)	PSPORT1
30	640-684	Hematopoietic/Immune (0.333) Cardiovascular (0.167) Endocrine (0.167)	Cancer (0.667) Inflammation (0.333) Fetal/Cell Proliferation (0.167)	pINCY
31	1028-1072	Cardiovascular (0.333) Nervous (0.333) Reproductive (0.333)	Inflammation (0.667) Cancer (0.333)	PSPORT1
32	271-315	Endocrine (0.500) Reproductive (0.500)	Cancer (1.000)	pINCY
33	205-249	Nervous (0.750) Gastrointestinal (0.250)	Cancer (1.000) Fetal/Cell Proliferation (0.500)	PSPORT1
34	21-65	Reproductive (0.400) Hematopoietic/Immune (0.200) Nervous (0.200)	Cancer (0.400) Fetal/Cell Proliferation (0.200) Inflammation (0.200)	pINCY
35	273-317	Nervous (1.000)	Nervous (1.000)	pINCY
36	131-175	Reproductive (0.333) Gastrointestinal (0.222) Cardiovascular (0.111)	Cancer (0.333) Inflammation (0.222) Fetal/Cell Proliferation (0.111)	pINCY
37	58 - 87 376 - 405	Reproductive (1.000)	Cancer (0.750) Inflammation (0.250)	PBLUESCRIPT
38	109 - 168 415 - 474	Reproductive (0.300) Cardiovascular (0.143) Nervous (0.138) Gastrointestinal (0.113)	Cancer (0.488) Inflammation (0.330) Cell Proliferation (0.172)	pINCY
39	809 - 868 1229 - 1288	Reproductive (0.625) Gastrointestinal (0.188)	Cancer (0.438) Inflammation (0.437) Cell Proliferation (0.125)	PSPORT1
40	243 - 302	Hematopoietic/Immune (0.727) Dermatologic (0.091) Gastrointestinal (0.091) Reproductive (0.091)	Inflammation (0.545) Cell Proliferation (0.360) Cancer (0.182)	PSPORT1

Table 3 (cont.)

Nucleotide SEQ ID NO:	Selected Fragments	Tissue Expression (Fraction of Total)	Disease or Condition (Fraction of Total)	Vector
41	459 - 518	Nervous (0.555) Endocrine (0.111) Gastrointestinal (0.111) Reproductive (0.111) Cardiovascular (0.111)	Cancer (0.500) Neurological (0.111)	pINCY
42	241 - 300	Gastrointestinal (1.000)	Cancer (0.500)	pINCY
43	757-801	Reproductive (0.289) Hematopoietic/Immune (0.140) Nervous (0.132)	Cancer (0.465) Inflammation (0.360) Cell Proliferation (0.123)	pINCY
44	165-209 434-479	Hematopoietic/Immune (1.000)	Cancer (0.300) Inflammation (0.300)	pINCY
45	1-46	Nervous (0.375) Reproductive (0.313) Gastrointestinal (0.093)	Cancer (0.281) Inflammation (0.313)	PSPORT1
46	866-910	Urologic (0.500) Nervous (0.250) Reproductive (0.250)	Cancer (0.750) Inflammation (0.250)	PSPORT1
47	1029-1073	Cardiovascular (0.234) Reproductive (0.221) Nervous (0.182)	Cancer (0.455) Inflammation (0.331) Cell proliferation (0.143)	pINCY
48	76-120	Hematopoietic/Immune (0.308) Gastrointestinal (0.231) Nervous (0.154)	Cancer (0.308) Cell proliferation (0.231) Inflammation (0.308)	pINCY
49	111-155	Reproductive (0.333) Cardiovascular (0.167) Developmental (0.167) Nervous (0.167)	Cancer (0.750) Cell proliferation (0.167) Inflammation (0.083)	pINCY
50	218-262	Nervous (1.000)	Cell proliferation (1.000)	PSPORT1
51	109-153	Dermatologic (0.500) Reproductive (0.500)		pINCY
52	277-321	Nervous (1.000)	Neurological (1.000)	pINCY

Table 4

Nucleotide SEQ ID NO:	Library	Library Description
27	BRAINOT11	This library was constructed using RNA isolated from brain tissue removed from the right temporal lobe of a 5-year-old Caucasian male during a hemispherectomy. Pathology indicated extensive polymicrogyria and mild to moderate gliosis (predominantly subpial and subcortical), consistent with chronic seizure disorder. Family history included a cervical neoplasm.
28	PANCNOT07	This library was constructed using RNA isolated from the pancreatic tissue of a Caucasian male fetus, who died at 23 weeks' gestation.
29	PROSNON01	This normalized prostate library was constructed from 4.4 M independent clones from a prostate library. Starting RNA was made from prostate tissue removed from a 28-year-old Caucasian male who died from a self-inflicted gunshot wound. The normalization and hybridization conditions were adapted from Soares, M.B. et al. (1994) Proc. Natl. Acad. Sci. USA 91:9228-9232, using a longer (19 hour) reannealing hybridization period.
30	SPLNNOT04	This library was constructed using RNA isolated from the spleen tissue of a 2-year-old Hispanic male, who died from cerebral anoxia.
31	HEALDIT02	This library was constructed using RNA isolated from diseased left ventricle tissue removed from a 56-year-old male during a heart transplant. Patient history included cardiovascular disease and myocardial infarction.
32	PROSTUT12	This library was constructed using RNA isolated from prostate tumor tissue removed from a 65-year-old Caucasian male during a radical prostatectomy. Pathology indicated an adenocarcinoma (Gleason grade 2+2). Adenofibromatous hyperplasia was also present. The patient presented with elevated prostate specific antigen (PSA).
33	BRAINON01	This library was constructed and normalized from 4.88 million independent clones from a brain library. RNA was made from brain tissue removed from a 26-year-old Caucasian male during cranioplasty and excision of a cerebral meningeal lesion. Pathology for the associated tumor tissue indicated a grade 4 oligoastrocytoma in the right fronto-parietal part of the brain.
34	BRSTTUT16	This library was constructed using RNA isolated from breast tumor tissue removed from a 43-year-old Caucasian female during a unilateral extended simple mastectomy. Pathology indicated recurrent grade 4, nuclear grade 3, ductal carcinoma. Angiolymphatic space invasion was identified. Left breast needle biopsy indicated grade 4 ductal adenocarcinoma. Paraffin embedded tissue was estrogen positive. Patient history included breast cancer and deficiency anemia. Family history included cervical cancer.

Table 4 (cont.)

Nucleotide SEQ ID NO:	Library	Library Description
35	BRADDIT02	This library was constructed using RNA isolated from diseased choroid plexus tissue of the lateral ventricle removed from the brain of a 57-year-old Caucasian male, who died from a cerebrovascular accident. Patient history included Huntington's disease, and emphysema.
36	UTRSTMR01	This library was constructed using 1.5 micrograms of polyA RNA isolated from uterine myometrial tissue removed from a 41-year-old Caucasian female during a vaginal hysterectomy. The myometrium and cervix were unremarkable; the endometrium was secretory and contained fragments of endometrial polyps. Pathology for associated tumor tissue indicated uterine leiomyoma. Patient history included ventral hernia and a benign ovarian neoplasm.
37	TESTNOT03	The library was constructed using RNA isolated from testicular tissue removed from a 37-year-old Caucasian male, who died from liver disease. Patient history included cirrhosis, jaundice, and liver failure.
38	SINTFET03	The library was constructed using RNA isolated from small intestine tissue removed from a Caucasian female fetus, who died at 20 weeks' gestation.
39	UTRSNOT02	The library was constructed using RNA isolated from uterine tissue removed from a 34-year-old Caucasian female during a vaginal hysterectomy. Patient history included mitral valve disorder. Family history included stomach cancer, congenital heart anomaly, irritable bowel syndrome, ulcerative colitis, colon cancer, cerebrovascular disease, type II diabetes, and depression.
40	THYMNON04	The normalized thymus library was constructed using RNA isolated from thymus tissue removed from a 3-year-old Caucasian male, who died from anoxia.
41	TESTNOT07	The library was constructed using RNA isolated from testicular tissue removed from a 31-year-old Caucasian male during an unilateral orchiectomy (excision of testis). Pathology indicated a mass containing a large subcapsular hematoma with laceration of the tunica albuginea. The surrounding testicular parenchyma was extensively necrotic. The patient presented with a trunk injury.
42	LIVRTUT13	The library was constructed using RNA isolated from liver tumor tissue removed from a 62-year-old Caucasian female during partial hepatectomy and exploratory laparotomy. Pathology indicated metastatic intermediate grade neuroendocrine carcinoma, consistent with islet cell tumor, forming nodules ranging in size, in the lateral and medial left liver lobe. The pancreas showed fibrosis, chronic inflammation and fat necrosis consistent with pseudocyst. The gallbladder showed mild chronic cholecystitis. Patient history included malignant neoplasm of the pancreas tail, pulmonary embolism, hyperlipidemia, thrombophlebitis, joint pain in multiple joints, type II diabetes, benign hypertension, and cerebrovascular disease. Family history included pancreas cancer, secondary liver cancer, benign hypertension, and hyperlipidemia.

Table 4 (cont.)

Nucleotide SEQ ID NO:	Library	Library Description
43	LUNGFET03	Library was constructed using RNA isolated from lung tissue removed from a Caucasian female fetus, who died at 20 weeks' gestation.
44	TLYMNOT08	Library was constructed using RNA isolated from anergic/allogenic T-lymphocyte tissue removed from an adult (40-50-year-old) Caucasian male. The cells were incubated for 3 days in the presence of OKT3 mAb (1 microgram/ml OKT3) and 5% human serum.
45	UTRSNOT02	Library was constructed using RNA isolated from uterine tissue removed from a 34-year-old Caucasian female during a vaginal hysterectomy. Patient history included mitral valve disorder. Family history included stomach cancer, congenital heart anomaly, irritable bowel syndrome, ulcerative colitis, colon cancer, cerebrovascular disease, type II diabetes, and depression.
46	PROSTUT05	Library was constructed using RNA isolated from prostate tumor tissue removed from a 69-year-old Caucasian male during a radical prostatectomy. Pathology indicated adenocarcinoma (Gleason grade 3+4). Adenofibromatous hyperplasia was also present. Family history included congestive heart failure, multiple myeloma, hyperlipidemia, and rheumatoid arthritis.
47	LUNGNOT12	Library was constructed using RNA isolated from lung tissue removed from a 78-year-old Caucasian male during a segmental lung resection and regional lymph node resection. Pathology indicated fibrosis pleura was puckered, but not invaded. Pathology for the associated tumor tissue indicated an invasive pulmonary grade 3 adenocarcinoma. Patient history included cerebrovascular disease, arteriosclerotic coronary artery disease, thrombophlebitis, chronic obstructive pulmonary disease, and asthma. Family history included intracranial hematoma, cerebrovascular disease, arteriosclerotic coronary artery disease, and type I diabetes.
48	THYRNOT03	Library was constructed using RNA isolated from thyroid tissue removed from the left thyroid of a 28-year-old Caucasian female during a complete thyroidectomy. Pathology indicated a small nodule of adenomatous hyperplasia present in the left thyroid. Pathology for the associated tumor tissue indicated dominant follicular adenoma, forming a well-encapsulated mass in the left thyroid.
49	PROSTUT08	Library was constructed using RNA isolated from prostate tumor tissue removed from a 60-year-old Caucasian male during radical prostatectomy and regional lymph node excision. Pathology indicated an adenocarcinoma (Gleason grade 3+4). Adenofibromatous hyperplasia was also present. Patient history included a kidney cyst, and hematuria. Family history included tuberculosis, cerebrovascular disease, and arteriosclerotic coronary artery disease.



Table 4 (cont.)

Nucleotide SEQ ID NO:	Library	Library Description
50	HNT2AZS07	This subtracted library was constructed from RNA isolated from an hNT2 cell line (derived from a human teratocarcinoma that exhibited properties characteristic of a committed neuronal precursor) treated for three days with 0.35 micromolar AZ. The hybridization probe for subtraction was derived from a similarly constructed library from untreated hNT2 cells. 3.08M clones from the AZ-treated library were subjected to three rounds of subtractive hybridization with 3.04M clones from the untreated library. Subtractive hybridization conditions were based on the methodologies of Swaroop et al. (NAR (1991) 19:1954) and Bonaldo et al. (Genome Research (1996) 6:791).
51	SKIRNOT01	Library was constructed using RNA isolated from skin tissue removed from the breast of a 26-year-old Caucasian female during bilateral reduction mamoplasty.
52	BRABDIR01	Library was constructed using RNA isolated from diseased cerebellum tissue removed from the brain of a 57-year-old Caucasian male, who died from a cerebrovascular accident.

Table 5

Program	Description	Reference	Parameter Threshold
ABI FACTURA	A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
ABI/PARACEL FDF	A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA.	Mismatch <50%.
ABI AutoAssembler	A program that assembles nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
BLAST	A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx.	Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410; Altschul, S.F. et al. (1997) Nucleic Acids Res. 25: 3389-3402.	ESTs: Probability value= 1.0E-8 or less Full Length sequences: Probability value= 1.0E-10 or less
FASTA	A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises at least five functions: fasta, tfasta, fastx, tfastx, and ssearch.	Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad. Sci. 85:2444-2448; Pearson, W.R. (1990) Methods Enzymol. 183: 63-98; and Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489.	ESTs: fasta E value=1.0E-6 Assembled ESTs: fasta Identity= 95% or greater and Match length=200 bases or greater; fastx E value=1.0E-8 or less Full Length sequences: fastx score=100 or greater
BLIMPS	A BLOCKS IMProved Searcher that matches a sequence against those in BLOCKS, PRINTS, DOMO, PRODOM, and PFAM databases to search for gene families, sequence homology, and structural fingerprint regions.	Henikoff, S. and J.G. Henikoff, Nucl. Acid Res., 19:6565-72, 1991. J.G. Henikoff and S. Henikoff (1996) Methods Enzymol. 266:88-105; and Attwood, T.K. et al. (1997) J. Chem. Inf. Comput. Sci. 37: 417-424.	Score=1000 or greater; Ratio of Score/Strength = 0.75 or larger; and, if applicable, Probability value= 1.0E-3 or less
HMMER	An algorithm for searching a query sequence against hidden Markov model (HMM)-based databases of protein family consensus sequences, such as PFAM.	Krogh, A. et al. (1994) J. Mol. Biol., 235:1501-1531; Sonnhammer, E.L.L. et al. (1988) Nucleic Acids Res. 26:320-322.	Score=10-50 bits for PFAM hits, depending on individual protein families

Table 5 (cont.)

Program	Description	Reference	Parameter Threshold
ProfileScan	An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite.	Gribskov, M. et al. (1988) CABIOS 4:61-66; Gribskov, et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al. (1997) Nucleic Acids Res. 25: 217-221.	Normalized quality score ≥ GCG-specified "HGH" value for that particular Prosite motif. Generally, score=1.4-2.1.
Phred	A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability.	Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res. 8:186-194.	
Phrap	A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences.	Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M. S. Waterman (1981) J. Mol. Biol. 147:195-197; and Green, P., University of Washington, Seattle, WA.	Score= 120 or greater; Match length= 56 or greater
Consed	A graphical tool for viewing and editing Phrap assemblies	Gordon, D. et al. (1998) Genome Res. 8:195-202.	
SPScan	A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides.	Nielson, H. et al. (1997) Protein Engineering 10:1-6; Claverie, J.M. and S. Audic (1997) CABIOS 12: 431-439.	Score=3.5 or greater
Motifs	A program that searches amino acid sequences for patterns that matched those defined in Prosite.	Bairoch et al. <u>supra</u> ; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI.	

What is claimed is:

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
  - 5 a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-26.
  - b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-26.
  - c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-26, and
  - 10 d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-26.
2. An isolated polypeptide of claim 1 selected from the group consisting of SEQ ID NO:1-26.
- 15 3. An isolated polynucleotide encoding a polypeptide of claim 1.
4. An isolated polynucleotide of claim 3 selected from the group consisting of SEQ ID NO:27-52.
- 20 5. A recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide of claim 3.
6. A cell transformed with a recombinant polynucleotide of claim 5.
- 25 7. A transgenic organism comprising a recombinant polynucleotide of claim 5.
8. A method for producing a polypeptide of claim 1, the method comprising:
  - a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said
  - 30 cell is transformed with a recombinant polynucleotide, and said recombinant polynucleotide comprises a promoter sequence operably linked to a polynucleotide encoding the polypeptide of claim 1, and
  - b) recovering the polypeptide so expressed.
- 35 9. An isolated antibody which specifically binds to a polypeptide of claim 1.

10. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:

- a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:27-52,
- b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:27-52,
- c) a polynucleotide sequence complementary to a),
- d) a polynucleotide sequence complementary to b), and
- e) an RNA equivalent of a)-d).

11. An isolated polynucleotide comprising at least 60 contiguous nucleotides of a polynucleotide of claim 10.

12. A method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide of claim 10, the method comprising:

- a) hybridizing the sample with a probe comprising at least 16 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide, and
- b) detecting the presence or absence of said hybridization complex, and, optionally, if present, the amount thereof.

13. A method of claim 12, wherein the probe comprises at least 30 contiguous nucleotides.

14. A method of claim 12, wherein the probe comprises at least 60 contiguous nucleotides.

15. A pharmaceutical composition comprising an effective amount of a polypeptide of claim 1 and a pharmaceutically acceptable excipient.

16. A method for treating a disease or condition associated with decreased expression of functional EXCS, comprising administering to a patient in need of such treatment the pharmaceutical composition of claim 15.

17. A method for screening a compound for effectiveness as an agonist of a polypeptide of claim 1, the method comprising:

- a) exposing a sample comprising a polypeptide of claim 1 to a compound, and

b) detecting agonist activity in the sample.

18. A pharmaceutical composition comprising an agonist compound identified by a method of claim 17 and a pharmaceutically acceptable excipient.

5

19. A method for treating a disease or condition associated with decreased expression of functional EXCS, comprising administering to a patient in need of such treatment a pharmaceutical composition of claim 18.

10 20. A method for screening a compound for effectiveness as an antagonist of a polypeptide of claim 1, the method comprising:

- a) exposing a sample comprising a polypeptide of claim 1 to a compound, and
- b) detecting antagonist activity in the sample.

15 21. A pharmaceutical composition comprising an antagonist compound identified by a method of claim 20 and a pharmaceutically acceptable excipient.

20 22. A method for treating a disease or condition associated with overexpression of functional EXCS, comprising administering to a patient in need of such treatment a pharmaceutical composition of claim 21.

23. A method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a sequence of claim 4, the method comprising:

- 25
- a) exposing a sample comprising the target polynucleotide to a compound, and
  - b) detecting altered expression of the target polynucleotide.

1	M	A	A	L	Q	K	S	V	S	S	F	L	M	G	T	L	A	T	S	C	L	L	L	A	L	L	V	Q	G	5571181	
1	M	-	-	-	P	S	S	S	A	L	L	-	-	-	-	-	-	-	C	C	L	V	F	L	A	G	V	A	A	S	GI511295
1	M	-	-	-	H	S	S	-	A	L	L	-	-	-	-	-	-	-	C	C	L	V	L	L	T	G	V	R	A	S	GI1841298
1	M	-	-	-	H	S	S	-	A	L	L	-	-	-	-	-	-	-	C	C	L	V	L	L	T	G	V	R	A	S	GI106805
31	-	G	A	A	P	I	S	S	H	C	R	L	D	K	S	N	F	Q	Q	P	Y	I	T	N	R	T	-	F	M	5571181	
21	R	D	A	S	A	P	S	D	S	C	T	H	F	S	N	S	L	P	L	M	L	R	E	L	R	T	A	F	S	GI511295	
20	P	G	Q	G	T	Q	S	E	N	S	C	T	R	F	P	G	N	L	P	H	M	L	R	D	L	R	D	A	F	S	GI1841298
20	P	G	Q	G	T	Q	S	E	N	S	C	T	H	F	P	G	N	L	P	N	M	L	R	D	L	R	D	A	F	S	GI106805
59	L	A	K	E	A	S	L	A	D	N	N	T	D	V	R	L	I	G	E	K	L	F	H	G	V	S	M	S	E	R	5571181
51	R	V	K	N	F	F	Q	M	K	D	Q	L	D	S	M	L	L	T	Q	S	L	L	D	D	F	K	G	Y	L	G	GI511295
50	R	V	K	T	F	F	Q	M	K	D	Q	L	D	N	I	L	L	K	E	S	L	L	E	D	F	K	G	Y	L	G	GI1841298
50	R	V	K	T	F	F	Q	M	K	D	Q	L	D	N	L	L	L	K	E	S	L	L	E	D	F	K	G	Y	L	G	GI106805
89	C	Y	L	M	K	Q	V	L	N	F	T	L	E	E	V	L	F	P	Q	S	D	R	F	Q	P	Y	M	Q	E	V	5571181
81	C	Q	A	L	S	E	M	I	Q	F	Y	L	E	E	V	M	-	P	Q	A	E	N	H	G	P	E	I	K	E	H	GI511295
80	C	Q	A	L	S	E	M	I	Q	F	Y	L	E	E	V	M	-	P	Q	A	E	N	H	D	P	D	I	K	E	H	GI1841298
80	C	Q	A	L	S	E	M	I	Q	F	Y	L	E	E	V	M	-	P	Q	A	E	N	Q	D	P	D	I	K	A	H	GI106805
119	V	P	F	L	A	R	-	-	-	-	L	S	N	R	L	S	T	C	H	I	E	G	D	D	L	H	I	Q	R	N	5571181
110	V	N	S	L	G	E	N	L	K	T	L	R	L	R	L	R	R	C	H	R	F	L	P	C	E	N	K	S	K	A	GI511295
109	V	N	S	L	G	E	N	L	K	T	L	R	L	R	L	R	R	C	H	R	F	L	P	C	E	N	K	S	K	A	GI1841298
109	V	N	S	L	G	E	N	L	K	T	L	R	L	R	L	R	R	C	H	R	F	L	P	C	E	N	K	S	K	A	GI106805

FIGURE 1A

145	V	Q	K	L	K	D	T	V	K	K	L	G	E	S	G	E	I	K	A	I	G	E	L	D	L	L	-	-	-	-	5571181
140	V	E	Q	V	K	S	V	F	S	K	L	Q	E	R	G	V	Y	K	A	M	S	E	F	D	I	F	I	N	Y	I	GI511295
139	V	E	Q	V	K	N	A	F	S	K	L	Q	E	K	G	V	Y	K	A	M	S	E	F	D	I	F	I	N	Y	I	GI1841298
139	V	E	Q	V	K	N	A	F	N	K	L	Q	E	K	G	I	Y	K	A	M	S	E	F	D	I	F	I	N	Y	I	GI106805
171	-	-	-	F	M	S	L	R	N	A	C	I															5571181				
170	E	T	Y	T	T	M	K	M	K	-	N															GI511295					
169	E	A	Y	M	T	M	K	I	R	-	N															GI1841298					
169	E	A	Y	M	T	M	K	I	R	-	N															GI106805					

FIGURE 1B



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## SEQUENCE LISTING

&lt;110&gt; INCYTE GENOMICS, INC.

TANG, Y. Tom

YUE, Henry

LAL, Preeti

BURFORD, Neil

BANDMAN, Olga

BAUGHN, Mariah R.

AZIMZAI, Yalda

LU, Dyung Aina M.

PATTERSON, Chandra

&lt;120&gt; EXTRACELLULAR SIGNALING MOLECULES

&lt;130&gt; PF-0701 PCT

&lt;140&gt; To Be Assigned

&lt;141&gt; Herewith

&lt;160&gt; 55

&lt;170&gt; PERL Program

&lt;210&gt; 1

&lt;211&gt; 77

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 1288847CD1

&lt;400&gt; 1

Met	Gly	Lys	Glu	Trp	Val	Lys	Ile	Leu	Leu	Phe	Leu	Leu	His	Leu
1				5				10					15	
Ser	Asn	Phe	Phe	Thr	Ile	Val	Thr	Phe	Leu	Gly	Ser	Gln	Gly	Leu
				20				25					30	
Leu	Gln	Ser	Pro	Ser	Tyr	Glu	Lys	Leu	Val	Gly	Cys	Cys	Leu	Met
				35				40					45	
Thr	Arg	Gly	Cys	Phe	Ser	Pro	Ser	Val	Met	Leu	Pro	Ser	Ala	Ala
				50				55					60	
Pro	Ser	Gln	Gln	Asp	Ser	Pro	Ser	His	Ser	Arg	Ala	Pro	Gly	Pro
				65				70					75	

Cys Ser

&lt;210&gt; 2

&lt;211&gt; 88

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 1329044CD1

&lt;400&gt; 2

Met	Lys	Thr	Pro	Asn	Asp	Leu	Phe	Leu	Arg	Gln	Leu	Gly	Tyr	Leu
1				5				10					15	
Ser	Ile	Cys	Cys	Phe	Val	Phe	Ser	Ser	Glu	Glu	Ser	Lys	Asn	Tyr
				20				25					30	
Lys	Ile	Ser	Leu	Ile	Val	Tyr	Leu	Thr	Phe	Leu	Thr	Met	Glu	Thr
				35				40					45	

Lys Pro Arg Asn Ser Ile Tyr Ser Val Leu Thr Gln Ser Thr His

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	50		55		60
Pro Asp Phe Glu Ser	Pro Arg Thr Gly Val	Pro Asn Pro Arg Ala			
	65		70		75
Glu Asp Gln Tyr Gln	Phe Glu Ala Tyr Tyr	Arg Val Thr			
	80		85		

<210> 3  
 <211> 96  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> Incyte ID No: 1493630CD1

<400> 3	
Met Ser Met Gln Phe Leu Phe Lys Met Val Ala Leu Cys Cys Cys	
1	5 10 15
Leu Trp Lys Ile Ser Gly Cys Glu Glu Val Pro Leu Thr Tyr Asn	
	20 25 30
Leu Leu Lys Cys Leu Leu Asp Lys Ala His Cys Val Leu Leu Thr	
	35 40 45
Pro Cys Gly Tyr Ile Phe Ser Leu Ile Ser Pro Glu Ile Leu Lys	
	50 55 60
Leu Thr Leu Ile Thr Leu Gln Ile Leu Ile Leu Lys Asn Leu	
	65 70 75
His Leu Leu Trp Leu Thr Val Ser Ser Arg Cys Val His Arg Ser	
	80 85 90
Ser Ala Arg Lys Glu Lys	
	95

<210> 4  
 <211> 104  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> Incyte ID No: 1533041CD1

<400> 4	
Met Arg Leu Ser Leu Pro Leu Gly Ser Leu Leu Trp Pro Phe Leu	
1	5 10 15
Val Cys Gly Cys Leu Leu Gln Val Ala Leu Cys Gln Thr Arg Ser	
	20 25 30
Ala Pro His Leu Asp Thr His Ser Pro Val Ala Phe Gln Cys Ser	
	35 40 45
Gly Arg Lys Pro Val Ser Leu Asp Val Lys Leu Thr Leu Met Gly	
	50 55 60
Trp Gly Arg Gly Leu Gly Arg Arg Gly Arg Gly Glu Gly Thr	
	65 70 75
Glu Leu Arg Ile Ser Trp Ser Ala Leu Gln Ala Gln Arg Arg Ser	
	80 85 90
Ala Lys Val Leu Asn Arg Phe Ser Leu Glu Ile Lys Asn Pro	
	95 100

<210> 5  
 <211> 60  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> Incyte ID No: 1566162CD1

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&lt;400&gt; 5

Met	Leu	Met	Phe	Ile	Lys	Gly	Leu	Ser	Ser	Thr	Leu	Phe	Leu	Gly
1				5					10					15
Ser	Thr	Leu	Ser	His	Arg	Asp	Pro	Ile	Cys	Phe	Tyr	Ser	Phe	His
				20					25					30
Phe	His	Leu	Tyr	Leu	Leu	Pro	His	Ala	Val	Ser	Pro	Val	Thr	Asn
				35					40					45
Ser	Ile	Tyr	Asn	Tyr	Leu	Leu	Gly	Leu	Tyr	Leu	Asp	Thr	Cys	Thr
				50					55					60

&lt;210&gt; 6

&lt;211&gt; 117

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 1811831CD1

&lt;400&gt; 6

Met	Pro	Lys	Ser	Gln	Ser	His	His	Leu	Thr	Gln	Leu	Gln	Leu	Leu
1				5					10					15
Pro	Ser	Cys	Leu	Leu	Gly	Leu	Leu	Pro	Pro	Val	Pro	Ala	Val	His
				20					25					30
Ala	Tyr	Ile	Leu	Gln	Gly	Cys	Val	Leu	Ser	Gly	Arg	Glu	Ile	Phe
				35					40					45
Phe	Ser	Val	Leu	Gln	Phe	Phe	Thr	Gln	Thr	Phe	Ser	Phe	Val	Val
				50					55					60
Pro	Val	Phe	Pro	Ser	Phe	Pro	Gly	Gly	Phe	Arg	Leu	Pro	Phe	Ser
				65					70					75
Ser	Pro	Trp	Leu	Ser	Leu	Cys	Pro	Ile	His	Arg	Ser	Thr	Leu	Gln
				80					85					90
Ala	Cys	Leu	Tyr	Glu	Arg	Gly	Leu	Phe	Leu	Cys	Arg	Lys	Leu	Thr
				95					100					105
Leu	Thr	Arg	Cys	Gly	Cys	Ser	Tyr	Thr	Asp	Leu	Ile			
				110					115					

&lt;210&gt; 7

&lt;211&gt; 86

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 1835447CD1

&lt;400&gt; 7

Met	Arg	Ala	Lys	Gly	Phe	Leu	Ala	Pro	Ser	Leu	Val	Leu	Ala	Val
1				5					10					15
Ser	Leu	Glu	Leu	Met	His	Pro	Asp	Ala	Asn	Ser	Pro	Ser	Glu	Cys
				20					25					30
Arg	Gly	Asp	Glu	Thr	Leu	Thr	Gly	Gln	Phe	Asn	Leu	Tyr	Met	Gly
				35					40					45
Asp	Lys	Leu	Glu	Gly	Lys	Thr	Asn	Gly	Arg	Arg	Val	Lys	Arg	Lys
				50					55					60
Leu	Asn	Tyr	Cys	Ala	Asn	Thr	Arg	His	Ser	Asn	Pro	Gly	Gly	Tyr
				65					70					75
Cys	Arg	Val	Asn	Asn	Asp	Arg	Tyr	Tyr	Phe	Val				
				80					85					

&lt;210&gt; 8

&lt;211&gt; 109

&lt;212&gt; PRT



<400> 12  
Met Glu Gly Pro Glu Phe Leu Arg Thr Ala Thr Ser Ala Ser Gly  
1 5 10 15

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```

Arg Gly Glu His Arg Ala Glu Gly Val Cys Ser Arg Leu Arg Glu
      20      25      30
Ala Ala Arg Arg Arg Gly Arg Pro Ser Leu Lys Gly Lys Arg Lys
      35      40      45
Arg Gly Ser Ala Ser Ile Pro Glu Arg Gly Leu Gly Arg Met Lys
      50      55      60
Thr Ser Ala Glu Leu His Glu Gln Glu Lys Pro Pro Ser Ser Pro
      65      70      75
Arg Ala Thr Gly Pro Gly Arg Leu Gly His Ala Arg Gly Arg Gly
      80      85      90
Pro Asp Ala Leu Arg Gly Gly Ala Ala Gly Pro Gly Arg Ala Ser
      95     100     105
Ser Gly Ala Pro Arg Glu Arg Lys Met Ala Pro His Gly Pro Gly
     110     115     120
Ser Leu Thr Thr Leu Val Pro Trp Ala Ala Ala Leu Leu Leu Ala
     125     130     135
Leu Gly Val Glu Arg Ala Leu Ala Leu Pro Glu Ile Cys Thr Gln
     140     145     150
Cys Pro Gly Ser Val Gln Asn Leu Ser Lys Val Ala Phe Tyr Cys
     155     160     165
Lys Thr Thr Arg Glu Leu Met Leu His Ala Arg Cys Cys Leu Asn
     170     175     180
Gln Lys Gly Thr Ile Leu Gly Leu Asp Leu Gln Asn Cys Ser Leu
     185     190     195
Glu Asp Pro Gly Pro Asn Phe His Gln Ala His Thr Thr Val Ile
     200     205     210
Ile Asp Leu Gln Ala Asn Pro Leu Lys Gly Asp Leu Ala Asn Thr
     215     220     225
Phe Arg Gly Phe Thr Gln Leu Gln Thr Leu Ile Leu Pro Gln His
     230     235     240
Val Asn Cys Pro Gly Gly Ile Asn Ala Trp Asn Thr Ile Thr Ser
     245     250     255
Tyr Ile Asp Asn Gln Ile Cys Gln Gly Gln Lys Asn Leu Cys Asn
     260     265     270
Asn Thr Gly Asp Pro Glu Met Cys Pro Glu Asn Gly Ser Cys Val
     275     280     285
Pro Asp Gly Pro Gly Leu Leu Gln Cys Val Cys Ala Asp Gly Phe
     290     295     300
His Gly Tyr Lys Cys Met Arg Gln Gly Ser Phe Ser Leu Leu Met
     305     310     315
Phe Phe Gly Ile Leu Gly Ala Thr Thr Leu Ser Val Ser Ile Leu
     320     325     330
Leu Trp Ala Thr Gln Arg Arg Lys Ala Lys Thr Ser
     335     340

```

&lt;210&gt; 13

&lt;211&gt; 451

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 2267403CD1

&lt;400&gt; 13

```

Met Val Pro Glu Val Arg Val Leu Ser Ser Leu Leu Gly Leu Ala
      1      5      10      15
Leu Leu Trp Phe Pro Leu Asp Ser His Ala Arg Ala Arg Pro Asp
      20      25      30
Met Phe Cys Leu Phe His Gly Lys Arg Tyr Ser Pro Gly Glu Ser
      35      40      45
Trp His Pro Tyr Leu Glu Pro Gln Gly Leu Met Tyr Cys Leu Arg

```

	50		55		60
Cys Thr Cys Ser	Glu Gly Ala His Val	Ser Cys Tyr Arg Leu	His		
	65		70		75
Cys Pro Pro Val	His Cys Pro Gln Pro	Val Thr Glu Pro Gln	Gln		
	80		85		90
Cys Cys Pro Lys	Cys Val Glu Pro His	Thr Pro Ser Gly Leu	Arg		
	95		100		105
Ala Pro Pro Lys	Ser Cys Gln His Asn	Gly Thr Met Tyr Gln	His		
	110		115		120
Gly Glu Ile Phe	Ser Ala His Glu Leu	Phe Pro Ser Arg Leu	Pro		
	125		130		135
Asn Gln Cys Val	Leu Cys Ser Cys Thr	Glu Gly Gln Ile Tyr	Cys		
	140		145		150
Gly Leu Thr Thr	Cys Pro Glu Pro Gly	Cys Pro Ala Pro Leu	Pro		
	155		160		165
Leu Pro Asp Ser	Cys Cys Gln Ala Cys	Lys Asp Glu Ala Ser	Glu		
	170		175		180
Gln Ser Asp Glu	Glu Asp Ser Val Gln	Ser Leu His Gly Val	Arg		
	185		190		195
His Pro Gln Asp	Pro Cys Ser Ser Asp	Ala Gly Arg Lys Arg	Gly		
	200		205		210
Pro Gly Thr Pro	Ala Pro Thr Gly Leu	Ser Ala Pro Leu Ser	Phe		
	215		220		225
Ile Pro Arg His	Phe Arg Pro Lys Gly	Ala Gly Ser Thr Thr	Val		
	230		235		240
Lys Ile Val Leu	Lys Glu Lys His Lys	Lys Ala Cys Val His	Gly		
	245		250		255
Gly Lys Thr Tyr	Ser His Gly Glu Val	Trp His Pro Ala Phe	Arg		
	260		265		270
Ala Phe Gly Pro	Leu Pro Cys Ile Leu	Cys Thr Cys Glu Asp	Gly		
	275		280		285
Arg Gln Asp Cys	Gln Arg Val Thr Cys	Pro Thr Glu Tyr Pro	Cys		
	290		295		300
Arg His Pro Glu	Lys Val Ala Gly Lys	Cys Cys Lys Ile Cys	Pro		
	305		310		315
Glu Asp Lys Ala	Asp Pro Gly His Ser	Glu Ile Ser Ser Thr	Arg		
	320		325		330
Cys Pro Lys Ala	Pro Gly Arg Val Leu	Val His Thr Ser Val	Ser		
	335		340		345
Pro Ser Pro Asp	Asn Leu Arg Arg Phe	Ala Leu Glu His Glu	Ala		
	350		355		360
Ser Asp Leu Val	Glu Ile Tyr Leu Trp	Lys Leu Val Lys Asp	Glu		
	365		370		375
Glu Thr Glu Ala	Gln Arg Gly Glu Val	Pro Gly Pro Arg Pro	His		
	380		385		390
Ser Gln Asn Leu	Pro Leu Asp Ser Asp	Gln Glu Ser Gln Glu	Ala		
	395		400		405
Arg Leu Pro Glu	Arg Gly Thr Ala Leu	Pro Thr Ala Arg Trp	Pro		
	410		415		420
Pro Arg Arg Ser	Leu Glu Arg Leu Pro	Ser Pro Asp Pro Gly	Ala		
	425		430		435
Glu Gly His Gly	Gln Ser Arg Gln Ser	Asp Gln Asp Ile Thr	Lys		
	440		445		450
Thr					

<210> 14  
<211> 189  
<212> PRT  
<213> Homo sapiens  
  
<220>

PF-0701 PCT

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 2933038CD1

&lt;400&gt; 14

```

Met Leu Gly Ser Arg Ala Val Met Leu Leu Leu Leu Pro Trp
 1                    5                10                15
Thr Ala Gln Gly Arg Ala Val Pro Gly Gly Ser Ser Pro Ala Trp
                20                25                30
Thr Gln Cys Gln Gln Leu Ser Gln Lys Leu Cys Thr Leu Ala Trp
                35                40                45
Ser Ala His Pro Leu Val Gly His Met Asp Leu Arg Glu Glu Gly
                50                55                60
Asp Glu Glu Thr Thr Asn Asp Val Pro His Ile Gln Cys Gly Asp
                65                70                75
Gly Cys Asp Pro Gln Gly Leu Arg Asp Asn Ser Gln Phe Cys Leu
                80                85                90
Gln Arg Ile His Gln Gly Leu Ile Phe Tyr Glu Lys Leu Leu Gly
                95                100               105
Ser Asp Ile Phe Thr Gly Glu Pro Ser Leu Leu Pro Asp Ser Pro
                110               115               120
Val Gly Gln Leu His Ala Ser Leu Leu Gly Leu Ser Gln Leu Leu
                125               130               135
Gln Pro Glu Gly His His Trp Glu Thr Gln Gln Ile Pro Ser Leu
                140               145               150
Ser Pro Ser Gln Pro Trp Gln Arg Leu Leu Leu Arg Phe Lys Ile
                155               160               165
Leu Arg Ser Leu Gln Ala Phe Val Ala Val Ala Ala Arg Val Phe
                170               175               180
Ala His Gly Ala Ala Thr Leu Ser Pro
                185

```

&lt;210&gt; 15

&lt;211&gt; 216

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 3216587CD1

&lt;400&gt; 15

```

Met Gly Ala Val Met Gly Thr Phe Ser Ser Leu Gln Thr Lys Gln
 1                    5                10                15
Arg Arg Pro Ser Lys Asp Lys Ile Glu Asp Glu Leu Glu Met Thr
                20                25                30
Met Val Cys His Arg Pro Glu Gly Leu Glu Gln Leu Glu Ala Gln
                35                40                45
Thr Asn Phe Thr Lys Arg Glu Leu Gln Val Leu Tyr Arg Gly Phe
                50                55                60
Lys Asn Glu Cys Pro Ser Gly Val Val Asn Glu Asp Thr Phe Lys
                65                70                75
Gln Ile Tyr Ala Gln Phe Phe Pro His Gly Asp Ala Ser Thr Tyr
                80                85                90
Ala His Tyr Leu Phe Asn Ala Phe Asp Thr Thr Gln Thr Gly Ser
                95                100               105
Val Lys Phe Glu Asp Phe Val Thr Ala Leu Ser Ile Leu Leu Arg
                110               115               120
Gly Thr Val His Glu Lys Leu Arg Trp Thr Phe Asn Leu Tyr Asp
                125               130               135
Ile Asn Lys Asp Gly Tyr Ile Asn Lys Glu Glu Met Met Asp Ile
                140               145               150
Val Lys Ala Ile Tyr Asp Met Met Gly Lys Tyr Thr Tyr Pro Val

```



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155	160	165
Leu Lys Glu Asp Thr Pro Arg Gln His Val Asp Val Phe Phe Gln		
170	175	180
Lys Met Asp Lys Asn Lys Asp Gly Ile Val Thr Leu Asp Glu Phe		
185	190	195
Leu Glu Ser Cys Gln Glu Asp Asp Asn Ile Met Arg Ser Leu Gln		
200	205	210
Leu Phe Gln Asn Val Met		
215		

&lt;210&gt; 16

&lt;211&gt; 178

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 5037143CD1

&lt;400&gt; 16

Met Ala Ala Ala Arg Leu Cys Leu Ser Leu Leu Leu Leu Ser Thr		
1	5	10
Cys Val Ala Leu Leu Leu Gln Pro Leu Leu Gly Ala Gln Gly Ala		
20	25	30
Pro Leu Glu Pro Val Tyr Pro Gly Asp Asn Ala Thr Pro Glu Gln		
35	40	45
Met Ala Gln Tyr Ala Ala Asp Leu Arg Arg Tyr Ile Asn Met Leu		
50	55	60
Thr Arg Pro Arg Cys Val Pro Gln Leu Gly Arg Glu Ile Pro Ala		
65	70	75
Pro Gly Thr Leu Gly Pro Leu His Ile Pro Gly His Thr Leu Ser		
80	85	90
Pro Ala Pro Ala Pro Ala Pro Ser Arg Pro Ala Leu Gly Lys Thr		
95	100	105
Gly His Leu Cys Ser Thr Gly Leu Asp Gln Cys Ala Leu Gly Lys		
110	115	120
Met Val Pro Thr Gly Arg Tyr Glu Thr Gly Gly Leu Ala Pro Gly		
125	130	135
His Ser Ala Cys Pro Cys Cys Leu Phe Pro Pro Arg Tyr Gly Lys		
140	145	150
Arg His Lys Glu Asp Thr Leu Ala Phe Ser Glu Trp Gly Ser Pro		
155	160	165
His Ala Ala Val Pro Arg Glu Leu Ser Pro Leu Asp Leu		
170	175	

&lt;210&gt; 17

&lt;211&gt; 177

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 1235265CD1

&lt;400&gt; 17

Met Glu Pro Gly Asn Arg Ser Leu Asn Pro His Lys Thr Lys His		
1	5	10
His Met Glu Cys Arg Val Thr Gly Arg Ala Glu Val Thr Ala Ser		
20	25	30
Arg Glu Gly Arg Gly Ala Cys Ala Trp Glu Cys Gly Ser Ser Arg		
35	40	45
Gly Pro Trp Gly Leu Leu Arg Tyr Thr Phe Ala Pro Val Arg Ala		
50	55	60

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```

Ser Arg Pro Trp Ala Cys Leu Pro Lys Gly Ser Leu Ser Gln Arg
      65              70              75
Pro Lys Leu Pro Pro Pro Val His Leu Pro Pro Lys Ser Ser Cys
      80              85              90
Pro Pro Arg Ala Gly Gly Gly Gly Ala Gln Gly Arg Gly Val Pro
      95              100             105
Cys Thr Tyr Leu Ser Pro Leu Ser His Ser Pro Lys Thr Phe Cys
      110             115             120
Thr Phe Leu Gln Gly Cys Pro Ser Gln Gln Phe Pro Ser Trp Leu
      125             130             135
Ile Lys Pro Ser Asp Trp Cys Cys Val Pro Ser Leu Trp Pro Leu
      140             145             150
Cys Gly Glu Arg Gly Leu Gln Gly Glu Glu Pro Gly Arg Asp Ser
      155             160             165
Gln Ala Ser Pro Trp Glu Gly Gly Ala Ser Arg Arg
      170             175

```

&lt;210&gt; 18

&lt;211&gt; 179

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 5571181CD1

&lt;400&gt; 18

```

Met Ala Ala Leu Gln Lys Ser Val Ser Ser Phe Leu Met Gly Thr
  1          5          10          15
Leu Ala Thr Ser Cys Leu Leu Leu Leu Ala Leu Leu Val Gln Gly
      20          25          30
Gly Ala Ala Ala Pro Ile Ser Ser His Cys Arg Leu Asp Lys Ser
      35          40          45
Asn Phe Gln Gln Pro Tyr Ile Thr Asn Arg Thr Phe Met Leu Ala
      50          55          60
Lys Glu Ala Ser Leu Ala Asp Asn Asn Thr Asp Val Arg Leu Ile
      65          70          75
Gly Glu Lys Leu Phe His Gly Val Ser Met Ser Glu Arg Cys Tyr
      80          85          90
Leu Met Lys Gln Val Leu Asn Phe Thr Leu Glu Glu Val Leu Phe
      95          100         105
Pro Gln Ser Asp Arg Phe Gln Pro Tyr Met Gln Glu Val Val Pro
      110         115         120
Phe Leu Ala Arg Leu Ser Asn Arg Leu Ser Thr Cys His Ile Glu
      125         130         135
Gly Asp Asp Leu His Ile Gln Arg Asn Val Gln Lys Leu Lys Asp
      140         145         150
Thr Val Lys Lys Leu Gly Glu Ser Gly Glu Ile Lys Ala Ile Gly
      155         160         165
Glu Leu Asp Leu Leu Phe Met Ser Leu Arg Asn Ala Cys Ile
      170         175

```

&lt;210&gt; 19

&lt;211&gt; 213

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 685374CD1

&lt;400&gt; 19

```

Met Ala Leu Leu Arg Lys Ser Tyr Ser Glu Pro Gln Leu Lys Gly

```

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1	5	10	15
Ile Val Thr Lys Leu Tyr Ser Arg Gln Gly Tyr His Leu Gln Leu			
20	25	30	
Gln Ala Asp Gly Thr Ile Asp Gly Thr Lys Asp Glu Asp Ser Thr			
35	40	45	
Tyr Thr Leu Phe Asn Leu Ile Pro Val Gly Leu Arg Val Val Ala			
50	55	60	
Ile Gln Gly Val Gln Thr Lys Leu Tyr Leu Ala Met Asn Ser Glu			
65	70	75	
Gly Tyr Leu Tyr Thr Ser Glu Leu Phe Thr Pro Glu Cys Lys Phe			
80	85	90	
Lys Glu Ser Val Phe Glu Asn Tyr Tyr Val Thr Tyr Ser Ser Met			
95	100	105	
Ile Tyr Arg Gln Gln Gln Ser Gly Arg Gly Trp Tyr Leu Gly Leu			
110	115	120	
Asn Lys Glu Gly Glu Ile Met Lys Gly Asn His Val Lys Lys Asn			
125	130	135	
Lys Pro Ala Ala His Phe Leu Pro Lys Pro Leu Lys Val Ala Met			
140	145	150	
Tyr Lys Glu Pro Ser Leu His Asp Leu Thr Glu Phe Ser Arg Ser			
155	160	165	
Gly Ser Gly Thr Pro Thr Lys Ser Arg Ser Val Ser Gly Val Leu			
170	175	180	
Asn Gly Gly Lys Ser Met Ser His Asn Glu Ser Thr Pro Val Arg			
185	190	195	
Ala Lys Glu Gly Leu Cys Asn Arg Thr Leu Pro Pro Gly Ala Val			
200	205	210	
Glu Phe Phe			

&lt;210&gt; 20

&lt;211&gt; 239

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 843193CD1

&lt;400&gt; 20

Met Ala Ile Cys Pro Leu His Ser Ala Gly Gln Val Ala Cys Pro			
1	5	10	15
His Tyr Ile His Leu Leu Thr Pro Leu Pro Trp Met Asp Gln Trp			
20	25	30	
Trp Cys His Pro Lys Gln Ile Asp Thr Ile Phe Pro Leu Val Thr			
35	40	45	
Ala Lys Gly Glu Asn His Pro Ser Pro Asn Phe Asn Gln Tyr Val			
50	55	60	
Arg Asp Gln Gly Ala Met Thr Asp Gln Leu Ser Arg Arg Gln Ile			
65	70	75	
Arg Glu Tyr Gln Leu Tyr Ser Arg Thr Ser Gly Lys His Val Gln			
80	85	90	
Val Thr Gly Arg Arg Ile Ser Ala Thr Ala Glu Asp Gly Asn Lys			
95	100	105	
Phe Ala Lys Leu Ile Val Glu Thr Asp Thr Phe Gly Ser Arg Val			
110	115	120	
Arg Ile Lys Gly Ala Glu Ser Glu Lys Tyr Ile Cys Met Asn Lys			
125	130	135	
Arg Gly Lys Leu Ile Gly Lys Pro Ser Gly Lys Ser Lys Asp Cys			
140	145	150	
Val Phe Thr Glu Ile Val Leu Glu Asn Asn Tyr Thr Ala Phe Gln			
155	160	165	

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Asn	Ala	Arg	His	Glu	Gly	Trp	Phe	Met	Ala	Phe	Thr	Arg	Gln	Gly
				170					175					180
Arg	Pro	Arg	Gln	Ala	Ser	Arg	Ser	Arg	Gln	Asn	Gln	Arg	Glu	Ala
				185					190					195
His	Phe	Ile	Lys	Arg	Leu	Tyr	Gln	Gly	Gln	Leu	Pro	Leu	Thr	Asn
				200					205					210
His	Ala	Glu	Lys	Gln	Lys	Gln	Phe	Glu	Phe	Val	Gly	Ser	Ala	Pro
				215					220					225
Thr	Arg	Arg	Ala	Lys	Arg	Thr	Arg	Arg	Pro	Gln	Pro	Leu	Thr	
				230					235					

&lt;210&gt; 21

&lt;211&gt; 493

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 1359783CD1

&lt;400&gt; 21

Met	Leu	Lys	Ala	Leu	Phe	Leu	Thr	Met	Leu	Thr	Leu	Ala	Leu	Val
1				5					10					15
Lys	Ser	Gln	Asp	Thr	Glu	Glu	Thr	Ile	Thr	Tyr	Thr	Gln	Cys	Thr
				20					25					30
Asp	Gly	Tyr	Glu	Trp	Asp	Pro	Val	Arg	Gln	Gln	Cys	Lys	Asp	Ile
				35					40					45
Asp	Glu	Cys	Asp	Ile	Val	Pro	Asp	Ala	Cys	Lys	Gly	Gly	Met	Lys
				50					55					60
Cys	Val	Asn	His	Tyr	Gly	Gly	Tyr	Leu	Cys	Leu	Pro	Lys	Thr	Ala
				65					70					75
Gln	Ile	Ile	Val	Asn	Asn	Glu	Gln	Pro	Gln	Gln	Glu	Thr	Gln	Pro
				80					85					90
Ala	Glu	Gly	Thr	Ser	Gly	Ala	Thr	Thr	Gly	Val	Val	Ala	Ala	Ser
				95					100					105
Ser	Met	Ala	Thr	Ser	Gly	Val	Leu	Pro	Gly	Gly	Gly	Phe	Val	Ala
				110					115					120
Ser	Ala	Ala	Ala	Val	Ala	Gly	Pro	Glu	Met	Gln	Thr	Gly	Arg	Asn
				125					130					135
Asn	Phe	Val	Ile	Arg	Arg	Asn	Pro	Ala	Asp	Pro	Gln	Arg	Ile	Pro
				140					145					150
Ser	Asn	Pro	Ser	His	Arg	Ile	Gln	Cys	Ala	Ala	Gly	Tyr	Glu	Gln
				155					160					165
Ser	Glu	His	Asn	Val	Cys	Gln	Asp	Ile	Asp	Glu	Cys	Thr	Ala	Gly
				170					175					180
Thr	His	Asn	Cys	Arg	Ala	Asp	Gln	Val	Cys	Ile	Asn	Leu	Arg	Gly
				185					190					195
Ser	Phe	Ala	Cys	Gln	Cys	Pro	Pro	Gly	Tyr	Gln	Lys	Arg	Gly	Glu
				200					205					210
Gln	Cys	Val	Asp	Ile	Asp	Glu	Cys	Thr	Ile	Pro	Pro	Tyr	Cys	His
				215					220					225
Gln	Arg	Cys	Val	Asn	Thr	Pro	Gly	Ser	Phe	Tyr	Cys	Gln	Cys	Ser
				230					235					240
Pro	Gly	Phe	Gln	Leu	Ala	Ala	Asn	Asn	Tyr	Thr	Cys	Val	Asp	Ile
				245					250					255
Asn	Glu	Cys	Asp	Ala	Ser	Asn	Gln	Cys	Ala	Gln	Gln	Cys	Tyr	Asn
				260					265					270
Ile	Leu	Gly	Ser	Phe	Ile	Cys	Gln	Cys	Asn	Gln	Gly	Tyr	Glu	Leu
				275					280					285
Ser	Ser	Asp	Arg	Leu	Asn	Cys	Glu	Asp	Ile	Asp	Glu	Cys	Arg	Thr
				290					295					300
Ser	Ser	Tyr	Leu	Cys	Gln	Tyr	Gln	Cys	Val	Asn	Glu	Pro	Gly	Lys

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	305		310		315
Phe Ser Cys Met	Cys Pro Gln Gly Tyr	Gln Val Val Arg Ser	Arg		
	320		325		330
Thr Cys Gln Asp	Ile Asn Glu Cys Glu	Thr Thr Asn Glu Cys	Arg		
	335		340		345
Glu Asp Glu Met	Cys Trp Asn Tyr His	Gly Gly Phe Arg Cys	Tyr		
	350		355		360
Pro Arg Asn Pro	Cys Gln Asp Pro Tyr	Ile Leu Thr Pro Glu	Asn		
	365		370		375
Arg Cys Val Cys	Pro Val Ser Asn Ala	Met Cys Arg Glu Leu	Pro		
	380		385		390
Gln Ser Ile Val	Tyr Lys Tyr Met Ser	Ile Arg Ser Asp Arg	Ser		
	395		400		405
Val Pro Ser Asp	Ile Phe Gln Ile Gln	Ala Thr Thr Ile Tyr	Ala		
	410		415		420
Asn Thr Ile Asn	Thr Phe Arg Ile Lys	Ser Gly Asn Glu Asn	Gly		
	425		430		435
Glu Phe Tyr Leu	Arg Gln Thr Ser Pro	Val Ser Ala Met Leu	Val		
	440		445		450
Leu Val Lys Ser	Leu Ser Gly Pro Arg	Glu His Ile Val Asp	Leu		
	455		460		465
Glu Met Leu Thr	Val Ser Ser Ile Gly	Thr Phe Arg Thr Ser	Ser		
	470		475		480
Val Leu Arg Leu	Thr Ile Ile Val Gly	Pro Phe Ser Phe			
	485		490		

&lt;210&gt; 22

&lt;211&gt; 121

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 1440015CD1

&lt;400&gt; 22

Met Ala Arg Arg	Ala Gly Gly Ala Arg	Met Phe Gly Ser	Leu Leu
1	5	10	15
Leu Phe Ala Leu	Leu Ala Ala Gly Val	Ala Pro Leu Ser	Trp Asp
	20	25	30
Leu Pro Glu Pro	Arg Ser Arg Ala Ser	Lys Ile Arg Val	His Ser
	35	40	45
Arg Gly Asn Leu	Trp Ala Thr Gly His	Phe Met Gly Lys	Lys Ser
	50	55	60
Leu Glu Pro Ser	Ser Pro Ser Pro Leu	Gly Thr Ala Pro	His Thr
	65	70	75
Ser Leu Arg Asp	Gln Arg Leu Gln Leu	Ser His Asp Leu	Leu Gly
	80	85	90
Ile Leu Leu Leu	Lys Lys Ala Leu Gly	Val Ser Ser Ala	Ala Pro
	95	100	105
His Pro Lys Ser	Ser Thr Gly Gly Cys	Trp Tyr Lys Tyr	Leu Gln
	110	115	120

Lys

&lt;210&gt; 23

&lt;211&gt; 116

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 1652885CD1

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&lt;400&gt; 23

Met	Val	Pro	Gln	Pro	Pro	Thr	Thr	Cys	Pro	Trp	Lys	Pro	Val	Pro
1				5					10					15
Ser	Pro	Cys	Asp	Leu	Arg	Val	Gln	Gly	Ile	Cys	Pro	Ser	Ser	Phe
			20						25					30
Pro	Asp	Thr	Pro	Leu	Ala	Gln	Glu	Glu	Asp	Ser	Glu	Pro	Leu	Pro
			35						40					45
Pro	Gln	Asp	Ala	Gln	Thr	Ser	Gly	Ser	Leu	Leu	His	Tyr	Leu	Leu
			50						55					60
Gln	Ala	Met	Glu	Arg	Pro	Gly	Arg	Ser	Gln	Ala	Phe	Leu	Phe	Gln
			65						70					75
Pro	Gln	Arg	Phe	Gly	Arg	Asn	Thr	Gln	Gly	Ser	Trp	Arg	Asn	Glu
			80						85					90
Trp	Leu	Ser	Pro	Arg	Ala	Gly	Glu	Gly	Leu	Asn	Ser	Gln	Phe	Trp
			95						100					105
Ser	Leu	Ala	Ala	Pro	Gln	Arg	Phe	Gly	Lys	Lys				
			110						115					

&lt;210&gt; 24

&lt;211&gt; 136

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 4003984CD1

&lt;400&gt; 24

Met	Gln	Arg	Trp	Thr	Leu	Trp	Ala	Ala	Ala	Phe	Leu	Thr	Leu	His
1				5					10					15
Ser	Ala	Gln	Ala	Phe	Pro	Gln	Thr	Asp	Ile	Ser	Ile	Ser	Pro	Ala
			20						25					30
Leu	Pro	Glu	Leu	Pro	Leu	Pro	Ser	Leu	Cys	Pro	Leu	Phe	Trp	Met
			35						40					45
Glu	Phe	Lys	Gly	His	Cys	Tyr	Arg	Phe	Phe	Pro	Leu	Asn	Lys	Thr
			50						55					60
Trp	Ala	Glu	Ala	Asp	Leu	Tyr	Cys	Ser	Glu	Phe	Ser	Val	Gly	Arg
			65						70					75
Lys	Ser	Ala	Lys	Leu	Ala	Ser	Ile	His	Ser	Trp	Glu	Glu	Asn	Val
			80						85					90
Phe	Val	Tyr	Asp	Leu	Val	Asn	Ser	Cys	Val	Pro	Gly	Ile	Pro	Ala
			95						100					105
Asp	Val	Trp	Thr	Gly	Leu	His	Asp	His	Arg	Gln	Val	Arg	Lys	Gln
			110						115					120
Trp	Pro	Leu	Gly	Pro	Leu	Gly	Ser	Ser	Ser	Gln	Asp	Ser	Ile	Leu
			125						130					135

Ile

&lt;210&gt; 25

&lt;211&gt; 176

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 4365383CD1

&lt;400&gt; 25

Met	Asn	Phe	Val	His	Thr	Ser	Arg	Lys	Val	Lys	Ser	Leu	Asn	Pro
1				5					10					15
Lys	Lys	Phe	Ser	Ile	His	Asp	Gln	Asp	His	Lys	Val	Leu	Val	Leu
			20						25					30

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```

Asp Ser Gly Asn Leu Ile Ala Val Pro Asp Lys Asn Tyr Ile Arg
      35                      40                      45
Pro Glu Ile Phe Phe Ala Leu Ala Ser Ser Leu Ser Ser Ala Ser
      50                      55                      60
Ala Glu Lys Gly Ser Pro Ile Leu Leu Gly Val Ser Lys Gly Glu
      65                      70                      75
Phe Cys Leu Tyr Cys Asp Lys Asp Lys Gly Gln Ser His Pro Ser
      80                      85                      90
Leu Gln Leu Lys Lys Glu Lys Leu Met Lys Leu Ala Ala Gln Lys
      95                      100                     105
Glu Ser Ala Arg Arg Pro Phe Ile Phe Tyr Arg Ala Gln Val Gly
      110                     115                     120
Ser Trp Asn Met Leu Glu Ser Ala Ala His Pro Gly Trp Phe Ile
      125                     130                     135
Cys Thr Ser Cys Asn Cys Asn Glu Pro Val Gly Val Thr Asp Lys
      140                     145                     150
Phe Glu Asn Arg Lys His Ile Glu Phe Ser Phe Gln Pro Val Cys
      155                     160                     165
Lys Ala Glu Met Ser Pro Ser Glu Val Ser Asp
      170                     175

```

&lt;210&gt; 26

&lt;211&gt; 134

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 5497814CD1

&lt;400&gt; 26

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Met Ser Val Leu Pro Leu Cys Val Leu Pro Leu Leu Leu Ala Ser
  1      5                      10                      15
Cys Ser His Leu Ser Thr Phe Leu Trp Pro Pro Ser Leu Ala Cys
      20                      25                      30
Cys Leu Glu Thr Leu Val Gly Ile Pro Phe Ser Arg His Arg Ser
      35                      40                      45
Leu Gly Leu Ile Pro Ala Pro Arg Cys Leu Pro Leu Pro Ala Ala
      50                      55                      60
Ile Pro Thr Ser Leu Cys Ser Pro Pro Phe His Ser Leu His Ser
      65                      70                      75
Leu Pro Arg Cys Pro Leu Leu Lys Val Leu Gly His Pro Gln Val
      80                      85                      90
Ala Trp Ser Arg Gln Gln Pro Leu His Phe Thr Ser Ala Asn Asp
      95                      100                     105
Arg His Leu Ser Lys Ala Cys Pro Gly Cys Ser Trp Tyr Ser Ser
      110                     115                     120
Asp Ser Leu Val Ala Phe Gln Arg Pro Phe Pro Ser Gly Leu
      125                     130

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&lt;210&gt; 27

&lt;211&gt; 2730

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 1288847CB1

&lt;400&gt; 27

```

cgggtaggaa gctcctctta gtactaagag acttcaagct tcttgcttta agtcctcacc 60
ctttacatta tctaattctt cagttttgat gctgatacct gccccggcc ctaccttagc 120
tctgtggcat tatatctcct ctctgggact cttcaacctg gtactccata cctcttgtgc 180

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```

cctctcactt taggcagctt gcactattct tgaatgaatg aagaattatt tcctcatttg 240
gaagtaggag ggactgaaga aattctcccc aggcactgtg ggactgagag tcctattccc 300
ctagtaatag gtcataattcc cctagtaata tgagttctca aagcctacat tcaggatctc 360
cctctaggat gtgatagatc tggtcctctc ccttgaacta cccctccaca cgctctagtc 420
ccttcaacct accggtctat taagtgggtg cttttctctc cttggagtg cccaatttta 480
tattctcagg ggccaaggct aggtctgcaa cctctgtctc ctgacagatt gggagccaca 540
ggtgcctaag tgggaaccag ggcatgggaa aggagtgggt caaaattctt ctctttctcc 600
tccacctctc aaacttcttc actatagtga ccttccctagg ctctcagggg ctccctcagt 660
ccccatccta tgagaaacta gtgggttgct gcctgatgac aaggggttgt ttcagccctt 720
cagtcatgct gccttctgct gctccctccc agcaggatc accctctcat tcccggtctc 780
ctgggcccctg ttcttaggat cagtggcagg gagaaacggg tatctctttt ctctcttcta 840
atthtcagta taacaaaaaa ttatcccagc atgagcacgg gcacgtgccc ttcaccccat 900
tccacccttg tccagcaag actgggatgg gtacaactga actggggtct tcctttacta 960
ccccttcta cactcagctc ccagacacag ggtaggagg gggactgctg gctactgcag 1020
agacccttgg ctatttgagt aacctaggat tagtgagaag gggcagaagg agatacaact 1080
ccactgcaag tggaggtttt tttctacaag agttttctgc ccaaggccac agccatccca 1140
ctctctgctt ccttgagatt caaaccaaag gctgtttttc tatgtttaaa gaaaaaaaaa 1200
agtaaaaaac aaacacaaca cctcacaagt tgtaactctt ggtccttctc tctctccttt 1260
tctcttccct tcttccctt tccatctttt tttccacatg tcctttcctt attggtctct 1320
ttacctccta cttttctcac tccctatcag ggatattttg gggggggatg gtaaaagggtg 1380
ggctaaggaa cagaccctgg gattagggcc ttaagggtct tgagaggagt ctacttgcc 1440
ttcttatggg aaggagacc ctaaaaaact tttctctctt tgtcctcctt tttctcccc 1500
actctgaggt tcccccaaga gaaccagatt ggcagggaga agcattgtgg ggcaattgtt 1560
cctccttgac aatgtagcaa taaatagatg ctgccaaggg cagaaaaatg ggaggttagc 1620
tcagagcaga gtagtctcta gagaaaggaa gaatcctcaa cggcaccttg ggtgcttagc 1680
tcctttttag aatgtcagca gagctgagat taatatctgg gcttttcttg aactattctg 1740
gttattgagc ccttctgtt agacctaccg cctccacct cttctgtgtc tgcgtgtgat 1800
ttggtgacac ttcataagga ctagtccctt ctggggtatc agagccttag ggtgccccca 1860
tccccttccc cagtcaactg tggcacctgt aacctcccgg aacatgaagg actatgctct 1920
gaggctatac tctgtgcccc tgagagcaga gactggaagg gcaagaccag gtgctaagga 1980
ggggagaggg ggcacctctg ctctctccag accatcactg cactttaacc aggtcttag 2040
gtacaaaatc ctacttttca gagccttcca gctctggaac ctcaaacatc ctcatgctct 2100
ctcccgctc cttttgcata aaaaaaaaaa taaagaaaaa gaaaaaaaaa tacacacaca 2160
ctgaaaccca catggagaaa agaggtgttt ccttttatat tgctattcaa aatcaatacc 2220
accaacaaaa tattttctaag tagacacttt tccagacctt tgtttttttg tgcagtgtc 2280
caagctgcag ataggatttt gtaatacttc tggcagcttc tttccttgtg tacataatat 2340
atatatatac atatataat atatttttaa tcagaagtta tgaagaacaa aaagaaaaaa 2400
taaacacaga agcaagtgca ataccacctc tcttctccct ctctcctagg gtttctttg 2460
tagcctatgt ttggtgtctc ttttgacctt tacccttca cctcctctc tcttctctg 2520
attccccctc ccccttttt taaagagttt tctcctttc tcaaggggag ttaaactagc 2580
ttttgagact tattgcaaag catthttgtat atgtaataa ttgtaagtaa atatttgtgt 2640
aacggagata tactactgta agttttgtac tgtactggct gaaagtctgt tataataaaa 2700
catgagtaat ttaacaccaa aaaaaaaaaa 2730

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&lt;210&gt; 28

&lt;211&gt; 1339

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 1329044CB1

&lt;400&gt; 28

```

cacacatttt gaaatatgtg tcaaatattt aggaatacta atttaattcta aaaatccata 60
attgaaattt aaagagttta aagtaacaaa aatagactga aaaattaatt atccaacaat 120
atgaaaaccc caaatgacct atttctcaga caactagggt atctttcaat ctgctgcttt 180
gtattttctt ctgaagagtc aaaaaattat aaaatatact taatagtcta cttgacattt 240
ttgactatgg aaaccaagcc caggaatagt atatacagtg tactaaccac gtcaaccac 300
ccagactttg agtccccacg tacaggagtc cccaacccca gggccgaaga ccagtaaccag 360
tttgaggcct attaccgggt cacatagcag gaggtgagca ggggatgag gcagcattac 420

```



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```
tgcctgaact ctgcctcctg ttagatcagc agcagcatta ggttctcata ggagtgtgaa 480
ccctattgtg aactgtgcag gtgagggatc taggttgcag gctccttatg agaatactaat 540
gcctgatgat ccgagtggga cagtttcatc ccaaaaccat ccaccaccac caccctctcc 600
catgtccatg gaaaaactgt ctttgacaaa actggctcct ggtgccaaaa aggttgggga 660
ccgctgccta agtagacca gtctatgagt atctaaaccc agcagacaaa gtacacattc 720
acatccaaaa aatgaagagc aggagtaaga tgagcagagc atcagtgagg ctttggaag 780
gtcaactatt agcatccatt ttgatatgct ggcatctcat tggatattgg acattcacag 840
catcattctc ttccagggaag ggagaagagc tgtggagtag atctgtgagc aaaagagaaa 900
tggaagacag tggctgatcc caaatacatt tgagtagcag ataattaaga agagttatac 960
aggccagaga caacgaacac aaagaatcta acagtctacc caaaaattat gccctaaac 1020
agtgacttct caaccagact caatttctct gcaatgtctg gagacattca gtagttgtct 1080
ggagacattc agtagttgtc acaactgagc tggaggtagt gtgttgctcc tggcatttag 1140
taggtagaga tcagggatgc tgctaaacac cctacagtgt acaggacagc ccctacaaca 1200
aagaattaac caaaatgtca acaatgctaa ggctgagaaa ctctgaccta aatgacaat 1260
cattatgact aacctgtgc atagctgaaa agatccatga aaagccttaa aatagatcgc 1320
aataaacatt atgtagtca 1339
```

&lt;210&gt; 29

&lt;211&gt; 987

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 1493630CB1

&lt;400&gt; 29

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aaatgtgcat agcagaatgt taacagacgc tgcctttagg gagagataaa aagcataatg 60
acattagcta ggaaagttaa ttttcagttc ttactgaagt gctgtatgaa actgaaattt 120
ccaaggaact gaattttgtg agccaaatga gcatgcaatt cttgtttaag atgggtggcct 180
tatgctgttg tctctggaag atctccggct gtgaggaagt cctctact tacaacctgc 240
tcaagtgcct cctagataaa gcgcactgtg tactcctgac acctgtggt tacatctttt 300
ccttgatcag tccagaaatt ctcaaactca ctttaatacac tttgcagatc ctcttaatac 360
tcaaaaatct acacttactg tggctgacag tttcaagcag atgtgttcat cgcagtagtg 420
caagaaaaga aaagtagaag aaccctgcag agatttgatg gaaccagct tctattcatt 480
aaaaccaatg gcaaaatata aagcaaatag gaggtgacga aggttacaaa gatacgtatt 540
gtttatgttt tccctgggggt gtgctgattg tcaggcatca gttccctgtg ccattcattc 600
cccaacacag catgcatcag aaattttatc aataaatgct ttctctctca atgttcaacc 660
tatgctgata gaccattaaa tacagttttt ggggttcacag cttgtcatca tcatttgtct 720
atacatgtgg aaaagaatat ctaataagat actctcagca ttttgacac ttaaactaag 780
atgctgaatg ctgtatttta cggaataatc agccacatta aatttggaga ctcaacaagc 840
atgctgtgaa cattcaacat taggtttaaa ttttattttt aaaagttaat aataaaagga 900
tatatgttaa gtattatgaa accctgcata tactgtaata aaatggtgga tgtgaatgga 960
caatatatgc aataaaatat ataaaaa 987
```

&lt;210&gt; 30

&lt;211&gt; 842

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 1533041CB1

&lt;400&gt; 30

```
ggtgcctcct gcagtttggg agacatggac cagcatctgg tcttgtttcc aggagcatag 60
aagccacatc gttgagacat caggaaggta aaaaccacgc ggcttagcca agccctaagc 120
ctgtccccag accaaccctg ggacctatac agaacagagg gccagagcta gggctgctgc 180
ttctgtctca gcccctttgc ctctgtctc ccatccctc aacaccctgc ttctccggg 240
gacgcttttg agtgggccct gcccggggag ctgcagagca gcagcacctt tctctgagaa 300
gaggtccttg gttgggtcaa ggacagggct gagcgtggaa gggggaggag tcaggggctc 360
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&lt;210&gt; 31

&lt;211&gt; 1125

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 1566162CB1

&lt;400&gt; 31

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&lt;210&gt; 32

&lt;211&gt; 597

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 1811831CB1

&lt;400&gt; 32

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<220>  
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<223> Incyte ID No: 3892281CB1

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<211> 996  
<212> DNA  
<213> Homo sapiens

<220>  
<221> misc\_feature  
<223> Incyte ID No: 4318494CB1

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&lt;210&gt; 36

&lt;211&gt; 795

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 5090841CB1

&lt;400&gt; 36

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&lt;210&gt; 37

&lt;211&gt; 1419

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

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&lt;223&gt; Incyte ID No: 2006548CB1

&lt;400&gt; 37

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&lt;210&gt; 38

&lt;211&gt; 1265

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 2207183CB1

&lt;400&gt; 38

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&lt;210&gt; 39

&lt;211&gt; 1720

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 2267403CB1

&lt;400&gt; 39

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&lt;211&gt; 1055

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 2933038CB1

&lt;400&gt; 40

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&lt;210&gt; 41

&lt;211&gt; 1379

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 3216587CB1

&lt;400&gt; 41

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&lt;210&gt; 42

&lt;211&gt; 702

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 5037143CB1

&lt;400&gt; 42

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&lt;210&gt; 43

&lt;211&gt; 1855

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 1235265CB1

&lt;400&gt; 43

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&lt;210&gt; 44

&lt;211&gt; 1132

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 5571181CB1

&lt;400&gt; 44

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&lt;210&gt; 45

&lt;211&gt; 1906

&lt;212&gt; DNA



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&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 685374CB1

&lt;400&gt; 45

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&lt;210&gt; 46

&lt;211&gt; 1803

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 843193CB1

&lt;400&gt; 46

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&lt;210&gt; 47

&lt;211&gt; 3053

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 1359783CB1

&lt;400&gt; 47

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&lt;211&gt; 560

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 1440015CB1

&lt;400&gt; 48

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&lt;211&gt; 613

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&lt;400&gt; 49

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aagaaggaga aactgatgaa gctggctgcc caaaaggaaat cagcacgccg gcccttcctc 360  
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cagcagccac tgcactttac ctctgccaat gaccgtcatc tctccaaggc ctgccctggc 360
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&lt;210&gt; 53

&lt;211&gt; 179

&lt;212&gt; PRT

<213> *Cervus elaphus*

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: gi511295

&lt;400&gt; 53

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20     25     30
Cys Thr His Phe Ser Asn Ser Leu Pro Leu Met Leu Arg Glu Leu
35     40     45
Arg Thr Ala Phe Ser Arg Val Lys Asn Phe Phe Gln Met Lys Asp
50     55     60
Gln Leu Asp Ser Met Leu Leu Thr Gln Ser Leu Leu Asp Asp Phe
65     70     75
Lys Gly Tyr Leu Gly Cys Gln Ala Leu Ser Glu Met Ile Gln Phe
80     85     90
Tyr Leu Glu Glu Val Met Pro Gln Ala Glu Asn His Gly Pro Glu
95     100    105
Ile Lys Glu His Val Asn Ser Leu Gly Glu Lys Leu Lys Thr Leu
110    115    120
Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu Pro Cys Glu Asn
125    130    135
Lys Ser Lys Ala Val Glu Gln Val Lys Ser Val Phe Ser Lys Leu
140    145    150
Gln Glu Arg Gly Val Tyr Lys Ala Met Ser Glu Phe Asp Ile Phe
155    160    165
Ile Asn Tyr Ile Glu Thr Tyr Thr Thr Met Lys Met Lys Asn
170    175

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&lt;210&gt; 54

&lt;211&gt; 193

&lt;212&gt; PRT

<213> *Macaca fascicularis*

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Genbank ID No: gi1841298

&lt;400&gt; 54

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Met His Ser Ser Ala Leu Leu Cys Cys Leu Val Leu Leu Thr Gly
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20     25     30
Thr Arg Phe Pro Gly Asn Leu Pro His Met Leu Arg Asp Leu Arg
35     40     45
Asp Ala Phe Ser Arg Val Lys Thr Phe Phe Gln Met Lys Asp Gln
50     55     60
Leu Asp Asn Ile Leu Leu Lys Glu Ser Leu Leu Glu Asp Phe Lys
65     70     75

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Gly	Tyr	Leu	Gly	Cys	Gln	Ala	Leu	Ser	Glu	Met	Ile	Gln	Phe	Tyr			
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Leu	Glu	Glu	Val	Met	Pro	Gln	Ala	Glu	Asn	His	Asp	Pro	Asp	Ile			
				95					100					105			
Lys	Glu	His	Val	Asn	Ser	Leu	Gly	Glu	Asn	Leu	Lys	Thr	Leu	Arg			
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Leu	Arg	Leu	Arg	Arg	Cys	His	Arg	Phe	Leu	Pro	Cys	Glu	Asn	Lys			
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Ser	Lys	Ala	Val	Glu	Gln	Val	Lys	Asn	Ala	Phe	Ser	Lys	Leu	Gln			
				140					145					150			
Glu	Lys	Gly	Val	Tyr	Lys	Ala	Met	Ser	Glu	Phe	Asp	Ile	Phe	Ile			
				155					160					165			
Asn	Tyr	Ile	Glu	Ala	Tyr	Met	Thr	Met	Lys	Ile	Arg	Asn	Unk	Unk			
				170					175					180			
Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk			
				185					190								

&lt;210&gt; 55

&lt;211&gt; 178

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Genbank ID No: gi106805

&lt;400&gt; 55

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				20					25					30			
Thr	His	Phe	Pro	Gly	Asn	Leu	Pro	Asn	Met	Leu	Arg	Asp	Leu	Arg			
				35					40					45			
Asp	Ala	Phe	Ser	Arg	Val	Lys	Thr	Phe	Phe	Gln	Met	Lys	Asp	Gln			
				50					55					60			
Leu	Asp	Asn	Leu	Leu	Leu	Lys	Glu	Ser	Leu	Leu	Glu	Asp	Phe	Lys			
				65					70					75			
Gly	Tyr	Leu	Gly	Cys	Gln	Ala	Leu	Ser	Glu	Met	Ile	Gln	Phe	Tyr			
				80					85					90			
Leu	Glu	Glu	Val	Met	Pro	Gln	Ala	Glu	Asn	Gln	Asp	Pro	Asp	Ile			
				95					100					105			
Lys	Ala	His	Val	Asn	Ser	Leu	Gly	Glu	Asn	Leu	Lys	Thr	Leu	Arg			
				110					115					120			
Leu	Arg	Leu	Arg	Arg	Cys	His	Arg	Phe	Leu	Pro	Cys	Glu	Asn	Lys			
				125					130					135			
Ser	Lys	Ala	Val	Glu	Gln	Val	Lys	Asn	Ala	Phe	Asn	Lys	Leu	Gln			
				140					145					150			
Glu	Lys	Gly	Ile	Tyr	Lys	Ala	Met	Ser	Glu	Phe	Asp	Ile	Phe	Ile			
				155					160					165			
Asn	Tyr	Ile	Glu	Ala	Tyr	Met	Thr	Met	Lys	Ile	Arg	Asn					
				170					175								

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
23 November 2000 (23.11.2000)

PCT

(10) International Publication Number  
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5/10, C07K 14/47, 16/18, A61K 38/17, A01K 67/027,  
G01N 33/50, C12Q 1/68

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60/157,508 4 October 1999 (04.10.1999) US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:  
US 60/144,270 (CIP)  
Filed on 15 July 1999 (15.07.1999)  
US 60/146,700 (CIP)  
Filed on 30 July 1999 (30.07.1999)  
US 60/157,508 (CIP)  
Filed on 4 October 1999 (04.10.1999)  
US 60/134,949 (CIP)  
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(81) Designated States (national): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW.

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Published:

— With international search report.

(88) Date of publication of the international search report:  
28 June 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/70049 A3

(54) Title: EXTRACELLULAR SIGNALING MOLECULES

(57) Abstract: The invention provides human extracellular signaling molecules (EXCS) and polynucleotides which identify and encode EXCS. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating or preventing disorders associated with expression of EXCS.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/13975

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C12N5/10 C07K14/47 C07K16/18 A61K38/17  
A01K67/027 G01N33/50 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K A61K A01K G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, STRAND, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL SEQUENCES [Online] Accession No. AA760907, 27 January 1998 (1998-01-27) R. STRAUSBERG: "EST; H. sapiens cDNA clone IMAGE:1287455" XP002148318 compare nt 2116-1648 of seq. 27 and nt 15-486 of AA760907	1-17,20, 23
A	--- US 5 585 087 A (LUSTIG KEVIN D ET AL) 17 December 1996 (1996-12-17) the whole document --- -/--	1-17,20, 23

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

26 September 2000

Date of mailing of the international search report

28.12.00

Name and mailing address of the ISA

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NL - 2280 HV Rijswijk  
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Authorized officer

Galli, I



# INTERNATIONAL SEARCH REPORT

Intern. Application No

PCT/US 00/13975

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WATSON, HOPKINS, ROBERTS, STEITZ &amp; WEINER:            "Molecular Biology of the Gene, 4th            Edition, ISBN0-8053-961-4"            1988 , BENJAMIN CUMMINGS , MENLO PARK, CA            XP002148377            page 975; table 25.3            -----</p>	<p>1-17,20,            23</p>

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 00/13975

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☒ Claims Nos.: 18,19,21,22  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-23 partly

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-23, partly

An isolated polypeptide comprising the amino acid sequence of seq. ID 1 or homologs, fragments or epitopes thereof.

Corresponding nucleic acids (seq. ID 27), pharmaceutical compositions, antibodies, therapeutic and screening applications.

2. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 2,28

3. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 3,29

4. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 4,30

5. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 5,31

6. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 6,32

7. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 7,33

8. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 8,34

9. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 9,35

10. Claims: 1-23, partly

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Idem as subject-matter 1, but limited to seq. IDs 10,36

11. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 11,37

12. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 12,38

13. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 13,39

14. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 14,40

15. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 15,41

16. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 16,42

17. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 17,43

18. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 18,44

19. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 19,45

20. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 20,46

21. Claims: 1-23, partly

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Idem as subject-matter 1, but limited to seq. IDs 21,47

22. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 22,48

23. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 23,49

24. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 24,50

25. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 25,51

26. Claims: 1-23, partly

Idem as subject-matter 1, but limited to seq. IDs 26,52

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 16 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition. Claims 19 and 22 are equally directed to methods of treatment, but, for reasons listed elsewhere, they have not been searched at all.

Continuation of Box I.2

Claims Nos.: 18,19,21,22

Claims 18,19,21,22 relate to agonists and antagonists of the polypeptide claimed, however without giving a true technical characterization. Moreover, no such compounds are defined in the application. In consequence, said claims are ambiguous and vague, and the subject-matter is not sufficiently disclosed and supported by the description according to Art. 5 and 6 PCT. No search can be performed for such purely speculative claims, the wording of which is, in fact, a mere recitation of the results to be achieved.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

### Information on patent family members

PCT/US 00/13975

Form PCT/ISA/210 (patent family annex) (July 1992)